# Hsp70 interaction with membrane lipids regulate cellular functions in health and disease

Zsolt Balogi[a,b], Gabrielle Multhoff[c], Thomas Kirkegaard Jensen[d], Emyr Lloyd-Evans[e], Tetsumori Yamashima[f], Marja Jäättelä[g], John L. Harwood[h,*] and László Vígh[i,*]

[a]Department of Biochemistry and Medical Chemistry, University of Pécs Medical School, Pécs, Hungary

[b]Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

[c]Center of Translational Cancer Research, Radiation ImmunoOncology Group, Campus Klinikum rechts der Isar, Technische Universität München, Munich, Germany

[d]Orphazyme A/S, Copenhagen, Denmark

[e]School of Biosciences, Sir Martin Evans Building, Cardiff University, Cardiff, UK

[f]Department of Psychiatry and Behavioral Science, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

[g]Apoptosis Department and Centre for Genotoxic Stress, Institute for Cancer Biology, Danish Cancer Society, Copenhagen, Denmark

[h]School of Biosciences, Cardiff University, Cardiff, UK

[i]Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

*Corresponding authors:

John. L. Harwood (Harwood@cardiff.ac.uk) and László Vígh (vigh@brc.hu)

**Abstract**

Beyond guarding the cellular proteome the major stress inducible heat shock protein Hsp70 has been shown to interact with lipids. Non-cytosolic Hsp70 stabilizes membranes during stress challenges and, in pathophysiological states, facilitates endocytosis, counteracts apoptotic mechanisms, sustains survival pathways or represents a signal that can be recognized by the immune system. Disease-coupled lipid-associated functions of Hsp70 may be targeted via distinct subcellular localizations of Hsp70 itself or its specific interacting lipids. With a special focus on interacting lipids, here we discuss localization-dependent roles of the membrane-bound Hsp70 in the context of its therapeutic potential, particularly in cancer and neurodegenerative diseases.

## 1. Introduction

The stress-inducible heat shock protein 70, HSPA1A or Hsp70.1 (Hsp70 hereafter) (Hageman and Kampinga 2009) is expressed at low or undetectable levels in unstressed, healthy cells. Upon different stresses its expression is rapidly induced through mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and stress-activated protein kinase (SAPK) signaling cascades, which activate heat shock factors (HSFs) (Dubois and Bensaude 1993, Morimoto 1993, Adler, Schaffer et al. 1995, Xie and Huang 2003). Hsp70 restores the balance of the cell's proteome by assisting in refolding of denatured proteins. Importantly, Hsp70 is frequently upregulated in disease states, including cancer. The tumor microenvironment, where cells are subjected to free radicals, acidosis, hypoxia and nutrient deprivation, and where high levels of mutant proteins are present, causes stressful conditions challenging cancer cells (Xie and Huang 2003). The resultant high levels of Hsp70 in various cancer cells (Santarosa, Favaro et al. 1997, Nanbu, Konishi et al. 1998) enhances cell growth, suppresses senescence and confers resistance to stress-induced apoptosis (Gabai, Yaglom et al. 2009).

Hsp70 is commonly known as a cytosolic molecular chaperone that translocates to the nucleus upon stress conditions (Nollen, Salomons et al. 2001). However, it has been documented that Hsp70 also localizes to the luminal side of the endosomal-lysosomal system (Nylandsted, Gyrd-Hansen et al. 2004) and to the plasma membrane (Multhoff, Botzler et al. 1995, Multhoff, Botzler et al. 1997), as well as to the extracellular space (Asea, Kraeft et al. 2000) in pathophysiological states, such as cancer. Importantly, the unusual localization of Hsp70 is associated with a series of tumor specific functions such as counteracting lysosomal membrane permeabilization (LMP) and subsequent lysosome-dependent cell death (Kirkegaard, Roth et al. 2010) or immunomodulatory and invasion promoting roles of cell surface and extracellular Hsp70 (Gehrmann, Marienhagen et al. 2005). Given that normal cells do not show these specific features, Hsp70 unusually localized in endosomes, lysosomes and at the extracellular side represents therapeutically targetable functions.

In fact, membrane association and lipid interactions have also been reported for several other members of the ubiquitous heat shock protein family, e.g. small heat shock proteins, Hsp60, Hsp70 and Hsp90, in different organisms (Torok, Horvath et al. 1997, Torok, Goloubinoff et al. 2001, Tsvetkova, Horvath et al. 2002, Horvath, Multhoff et al. 2008, Zhang, Wang et al. 2018). As an indication of a functional interplay between

Hsps and membranes, expression of Hsps is controlled by the physical state of the membrane through activation of the Rac1-mediated heat shock response (Horvath, Glatz et al. 1998, Vigh, Maresca et al. 1998, Balogh, Horvath et al. 2005, Nagy, Balogi et al. 2007, Vigh, Horvath et al. 2007, Gungor, Gombos et al. 2014). Following specific lipid changes, membrane reorganization and interaction of Hsps with cellular membranes stabilize membrane structure and function during stress challenges (Balogi, Torok et al. 2005, Balogi, Cheregi et al. 2008, Balogh, Maulucci et al. 2011, Balogh, Peter et al. 2013). Membrane-controlled initiation and stopping of the heat shock response has led to the concept of regulating heat shock protein expression by modulating the membrane's lipid phase through "membrane lipid therapy" (Torok, Crul et al. 2014, Escriba, Busquets et al. 2015). The heat shock protein co-inducer hydroximic acid derivatives, such as Bimoclomol and BGP-15, are small multi-target molecules that intercalate into membranes and stabilize their lipid rafts by modulating membrane composition and structure (Torok, Tsvetkova et al. 2003, Gombos, Crul et al. 2011). Several studies have shown beneficial effects of BGP-15 on various disease models (Crul, Toth et al. 2013). It is noted that such Hsp co-inducer compounds potentiate the response to a pre-existing stress without exhibiting effects in nonstressed environments. Dihydropyridine derivatives, another recently explored family of Hsp co-inducers, such as LA1011 and LA1044, improve the spatial learning and memory functions in wild type mice, and eliminate neurodegeneration by increasing dendritic spine density and reducing tau pathology and amyloid plaque formation in APPxPS1 double mutant mouse model of Alzheimer's disease (Kasza, Hunya et al. 2016, Roe, Wahab et al. 2018). Recently it was shown that binding of these dihydropyridines to Hsp90 compromises Hsp90's chaperone activity (Roe, Wahab et al. 2018), which consequently induces the heat shock response in diseased cells. Furthermore, xenohormetic plant compounds with a general beneficial effect on animals also induce Hsp expression, and therefore have been applied for the treatment of neurodevelopmental delay (Hooper, Hooper et al. 2010). Further modulators of Hsp expression with respect to neurological diseases have been described elsewhere (Penke, Bogar et al. 2018, Penke, Paragi et al. 2018).

## 2. Membrane crossing and post-translational modifications of HSP70

Despite the high therapeutic potential of Hsp70 – membrane interaction, the mechanism by which Hsp70, lacking a leader sequence, is capable of crossing the endosomal-lysosomal or the plasma membrane is not well understood. *In vitro* studies with reconstituted protein-lipid systems have unraveled a specific interaction between Hsp70 and phosphatidylserine (PS) (Arispe, Doh et al. 2002, Lamprecht, Gehrmann et al. 2018) and proposed that Hsp70 oligomers generate pores in the cell membrane (Arispe, Doh et al. 2004). PS indeed confers a negative charge to the cytosolic leaflet of the plasma membrane and also to the endosomal membrane, allowing the recruitment of proteins with strong or moderate positive charges, respectively (Yeung, Gilbert et al. 2008). More recently, it has been shown that a cluster of positively charged Lys and Arg residues (R533 to K601/K597) anchor Hsc70/ Hsp70 to the endosomal membrane, which enables entry of Hsc70/Hsp70-cargo complexes to endosomes through microautophagy (Morozova, Clement et al. 2016). Interestingly, this lipid interacting region has been identified to be important for other functions as well. Hsp70 is composed of a nucleotide-binding domain (NBD) and a substrate-binding domain (SBD), which are connected by a linker (Fig. 1A). The linker domain (aa 384-397) and a fraction (aa 557-641) of the helical lid subdomain (HLS) of SBD, which overlaps with the lipid interacting region (R533, R535, K569, K573, K589, K597 of human Hsp70), are involved in oligomerization (Aprile, Dhulesia et al. 2013, Nimmervoll, Chtcheglova et al. 2015) (Fig. 1B). More specifically, Morgner et al. identified Lys rich regions throughout the whole molecule, but mostly in the SBD (K108-K561/569), that direct Hsp70 monomers in an antiparallel orientation (Morgner, Schmidt et al. 2015). T504, K561, K568, K569 and K507, K512, K526 residues of the SBD in ATP and ADP bound state allow not only dimerization but also interaction with the co-chaperones Hsp40, Hsp90, HopGR and client proteins (Fig. 1C). Importantly, phosphorylation and acetylation of these residues stabilize protein-protein interactions and, therefore, they are likely to also affect lipid interactions of this region. Further, trimethylation of K561 of the Hsp70 family members by METTL21A methyltransferase alter the affinity of Hsp70 towards monomeric and fibrillar $\alpha$-synuclein (Jakobsson, Moen et al. 2013), and phosphorylation and methylation of HLS residues including K561 and Y611 are necessary for proper ubiquitination by E3 ubiquitin ligase CHIP (Zhang, Amick et al. 2015). Hsp70 is ubiquitinated at 12 out of its 39 Lys residues including K561 (Soss, Rose et al. 2015). Hot-spots of phosphorylation in Ssa1, the

yeast homologue of Hsp70, at T36-S38 and T492-S495-T499 are important for normal growth and survival (Beltrao, Albanese et al. 2012). A large number of multiple post-translational modification sites point to a combinatorial code for a specific function (Cloutier and Coulombe 2013). Overlapping patterns of motives and post-translational modifications, in particular in the SBD, imply tight regulation of interrelated or interfering Hsp70 functions such as substrate or lipid binding (Fig. 1). To dissect the impact of post-translational modifications on Hsp70 localization and function necessitates further in-depth studies using subcellular fractions that can then be rendered to a specific function.

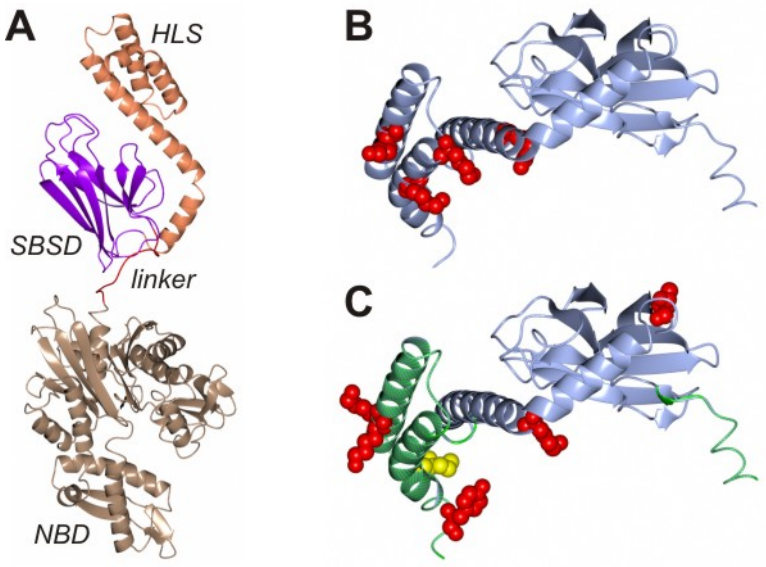


**Fig.1 Lipid interacting and post-translational modified regions of Hsp70 (A)** Full length crystal structure and domains of Hsp70 shown for the prokaryotic Hsp70 DnaK (PDB: 2KHO). Hsp70 has an N-terminal nucleotide binding domain (NBD: pale brown), a short linker region (red) that couples to the substrate binding domain (SBD) consisting of a substrate-binding subdomain (SBSD: purple) and a helical lid subdomain (HLS: coral). **(B)** Residues of the "lysine-arginine cluster" interacting with the lipid phosphatydilserine (PS) (PDB: 4PO2 of the linker and SBD of human Hsp70). Positively charged R533, R535, K569, K573, K589, K597 shown in red are proposed to specifically bind to PS at the cytoplasmic leaflet of endosomes, allowing Hsp70-cargo entry to endosomes via autophagy (Morozova, Clement et al. 2016). **(C)** Example residues that are post-translational modified (PTM) and functionally relevant (PDB: 4PO2 of the linker and SBD of human Hsp70). Regions involved in oligomerization of Hsp70 are shown in green. Further residues that are exposed to PTMs are shown (in red) as relevant for Hsp70 dimerization and client protein interaction (T504, K561, K568, K569 and K507, K512, K526), E3 Ub ligase CHIP interaction (K561, Y611). Different PTMs of K561 (in yellow) were

found to be important for substrate interaction, oligomerization, client and self-ubiquitination, cell growth and survival. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

## 3. HSP70 trafficking and tumor invasion

Cell surface, endosomal, lysosomal and extracellular pools of Hsp70 are interconnected in a highly dynamic fashion (Fig. 2). Plasma membrane-bound Hsp70 enters the endosomal route via clathrin dependent and independent mechanisms, and a fraction of internalized protein is recycled back to the surface. When excess Hsp70 is present in the cell, Hsp70 is further trafficked to late endosomes and lysosomes (Juhasz, Thuenauer et al. 2013). Cytosolic Hsp70 may also enter the endo-lysosomal system via an autophagic mechanism as implicated above (Morozova, Clement et al. 2016). Importantly, Hsp70 is resistant to proteolytic cleavage (and is, hence distinguishable from its cargos which are destined for lysosomal degradation) thus allowing it to exert its anti-apoptotic role. A large body of evidence describes Hsp70 present in both membrane-bound and soluble forms in the endo-lysosomal system, which are released by multivesicular bodies (Bausero, Gastpar et al. 2005, Gastpar, Gehrmann et al. 2005, Lancaster and Febbraio 2005, Cordonnier, Chanteloup et al. 2017) and secretory lysosomes (Mambula and Calderwood 2006, Juhasz, Thuenauer et al. 2013), respectively. These mechanisms not only supply plasma membrane bound Hsp70, but also result in a considerable amount of exosomal membrane-bound or soluble Hsp70 which has immunomodulatory potential.

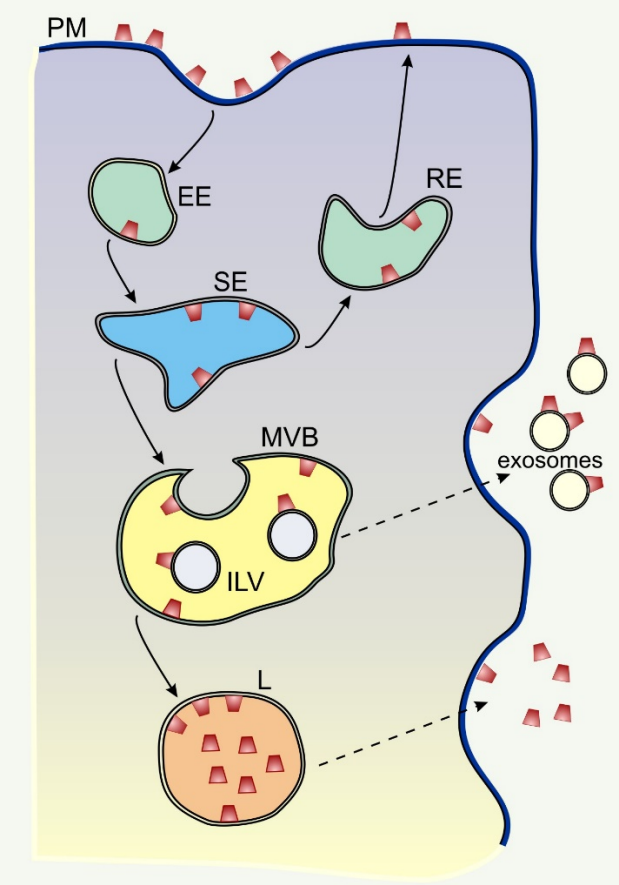


**Fig.2 Intracellular trafficking and secretion of Hsp70** Hsp70 (bucket symbol) is bound to the extracellular leaflet of the plasma membrane (PM). Surface Hsp70 is internalized to early endosomes (EE), a fraction of which is recycled back to the PM through sorting and recycling endosomes (SE, RE). Provided sufficient intracellular Hsp70, internalized Hsp70 is further trafficked to late endosomes/ multivesicular bodies (MVB), where BMP enriched intraluminal vesicles (ILV) are formed with Hsp70 attached to the membrane. Fusion of MVBs with the PM exposes Hsp70 at the cell surface and releases exosomes containing Hsp70. Hsp70 may further be targeted to lysosomes (L), which upon lysosomal exocytosis expose Hsp70 at the cell surface and release their soluble Hsp70 content to the extracellular space. Mechanisms of membrane crossing and supply of Hsp70 to the endolysosomal system are not known, but autophagy and direct membrane crossing mechanisms have been implicated.

Upregulated expression levels of Hsp70 is a diagnostic measure in several cancers, indicating increased cancer cell proliferation, ‘clinical stage’, or ‘increased grade’ together with shorter overall survival (Lazaris, Theodoropoulos et al. 1995, Kaur, Srivastava et al. 1998, Syrigos, Harrington et al. 2003, Juhasz, Lipp et al. 2013). Given the correlation between excess Hsp70 levels and its lysosomal, cell surface and extracellular appearance (Fig. 3), unusual localization of Hsp70 appears to be an attractive target for therapeutic interventions. These targets include but may not be limited to lysosomal membrane-bound Hsp70, which protects against lysosome-dependent cell death (Nylandsted, Gyrd-Hansen et al. 2004, Horvath and Vigh 2010, Kirkegaard, Roth et al. 2010), and plasma membrane- bound Hsp70, which promotes invasion (Gehrmann, Marienhagen et al. 2005, Murakami, Kuhnel et al. 2015) and endocytosis (Nimmervoll,

Chtcheglova et al. 2015, Chtcheglova and Hinterdorfer 2018). These features that would give rise to survival benefit for cancer patients may provide unique possibilities to fight tumor progression and metastasis. Moreover, surface localized and extracellular Hsp70 serve as potent stimuli for the innate immune system and can therefore be exploited as an effective adjuvant therapy (Multhoff, Pfister et al. 2000, Gong, Zhang et al. 2010). These targets and their therapeutic potential are detailed in the following sections.

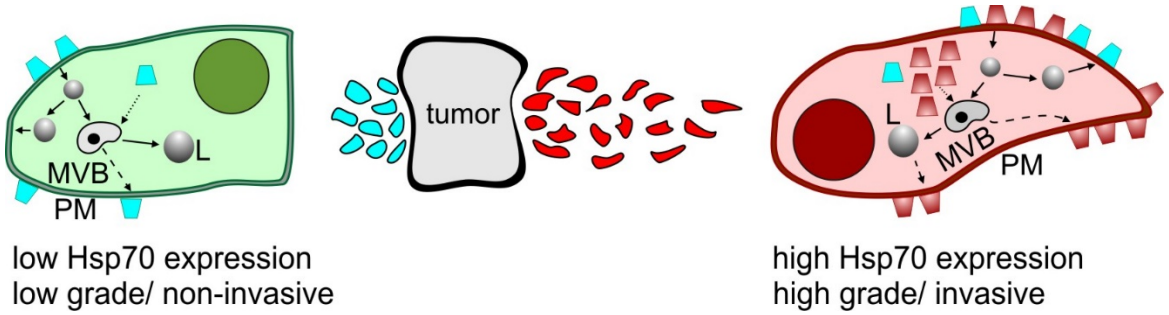


**Fig.3 Model for excess Hsp70-mediated tumor invasion** Low grade tumor cells with lower levels of intracellular Hsp70 (left side, bucket symbol used) also express low levels of Hsp70 in the endo-lysosomal system and at the cell surface, which correlate with a non-invasive phenotype. Contrary, high grade tumor cells often with high levels of intracellular Hsp70 (right side) express high levels of Hsp70 in the endo-lysosomal system and at the cell surface, as well as displaying an invasive phenotype. Anti-apoptotic effects of cytosolic or lysosomal Hsp70 and the tumor-promoting effect of surface Hsp70 are involved in facilitating tumor invasion as reviewed in (Juhasz, Lipp et al. 2013). Blue and red symbols correspond to basal (low level) and excess Hsp70, respectively. For trafficking routes refer to Fig. 2. MVB (late endosome, multivesicular body), L (lysosome), PM (plasma membrane). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

## 4. Plasma membrane bound and extracellular HSP70

Global cell surface protein profiling of membranes of tumor and normal cells revealed a tumor-specific, plasma membrane localization of a variety of different Hsps (Multhoff, Botzler et al. 1995, Shin, Wang et al. 2003, Tsuneki, Maruyama et al. 2013). Although lacking a classical consensual transmembrane sequence, Hsp70 also has been found on the cell surface (Multhoff, Botzler et al. 1995, Chen, Tao et al. 2002, Shin, Wang et al. 2003) and in the extracellular milieu of intact tumor cells (Pockley, Shepherd et al. 1998,

Pockley 2003, Calderwood, Mambula et al. 2007). Membrane localization of Hsps appears to be restricted to malignantly transformed cells (Multhoff, Botzler et al. 1995, Shin, Wang et al. 2003, Stangl, Gehrmann et al. 2011, Yang, Xu et al. 2015), bacterial/viral/fungal/parasite-infected cells and spermatogenic cells (Brown, Rixon et al. 2005, Bottger, Multhoff et al. 2012, Silveira, Piffer et al. 2013). In normal cells, Hsp70 is only found inside the cell but not on the plasma membrane. Therapeutic interventions such as radiochemotherapy, Hsp90 inhibition and hyperthermia have been found to further increase the levels of cytosolic and membrane-bound Hsps (Multhoff, Botzler et al. 1995, Gehrmann, Marienhagen et al. 2005, Zunino and Ricci 2016) in tumor cells. The presence of Hsp70 in the extracellular milieu of viable cells (Guzhova, Kislyakova et al. 2001, Triantafilou, Miyake et al. 2002, Barreto, Gonzalez et al. 2003) is currently explained by an alternative lysosomal/endosomal pathway (Fig. 2), which does not involve the classical ER-Golgi compartment (Mambula and Calderwood 2006). These findings concur with those from Asea and colleagues who demonstrated that drugs which perturb ER-Golgi transport, including monensin and brefeldin A, do not influence membrane expression and release of Hsp70 (Asea, Ara et al. 2001).

## 5. Membrane anchorage of HSPS in tumor cell membranes

Approximately 15 to 20 % of the total cellular Hsp70 is found on the plasma membrane of some tumor cells (Gehrmann, Liebisch et al. 2008). Since neither high-salt conditions nor changes in the extracellular pH affect the Hsp70 membrane expression density on tumor cells, it is unlikely that Hsp70 is bound to proteinous cell surface receptors (Theriault, Mambula et al. 2005). Already in 1989, Hightower and Guidon noted that Hsp71/Hsp73 could bind fatty acids and suggested possible direct interactions with membrane lipids (Hightower and Guidon 1989). Further on it has been proposed that Hsps accumulate in glycosphingolipid and cholesterol-rich microdomains (CRMs) (Uittenbogaard, Ying et al. 1998, Triantafilou, Miyake et al. 2002, Broquet, Thomas et al. 2003, Zech, Ejsing et al. 2009). CRMs were originally defined as regions within the plasma membrane that are enriched in cholesterol, glycosphingolipids, glycosylphosphatidylinositol-anchored proteins and some other acylated proteins (van Engeland, Nieland et al. 1998, Kishimoto, Ishitsuka et al. 2016). As super-resolution cell

imaging techniques are now suitable for investigating membrane lipid domains (Sonnino and Prinetti 2013, Sezgin, Levental et al. 2017) these early findings should be revisited. A more recent effort has confirmed strong binding of Hsp70 to cholesterol and sphingomyelin domains in model membranes, and importantly high resolution atomic force microscopy revealed nano-domain size (up to 200 nm in diameter) of Hsp70 clusters on the cellular membrane (see Fig.4). These results may point to possible Hsp70-membrane lipid platforms formed (Nimmervoll, Chtcheglova et al. 2015). “How these Hsp70 platforms are formed, and what is their role?” Glycosphingolipids that are enriched in tumor cell membranes, provide neoplastic and normal stem cell markers with immunogenic potential (Novak, Binnington et al. 2013). However, glycosphingolipid-mediated immunoreactivity is often limited by a cholesterol-induced reorientation of glycosphingolipid head groups in a parallel rather than perpendicular conformation, which in turn hinders their recognition by the immune system (Novak, Binnington et al. 2013). Therefore, one could assume that cholesterol depletion by methyl-beta-cyclodextrin might improve immunogenicity of tumor cells. A comparative lipidomic analysis of the glycosphingolipid content revealed significantly greater amounts of globotriaosylceramide Gb3 (Nutikka and Lingwood 2004) in tumor cells with a high compared to a low Hsp70 membrane expression. Gb3 is a receptor for Verotoxin (Lindberg, Brown et al. 1987, Lingwood, Law et al. 1987) and AB5-Shiga toxin, an enterotoxin produced by Shigella dysenteria and enterohemorrhagic *Escherichia coli*. It is frequently found in the plasma membrane of germinal center B cells and Burkitt`s lymphoma cells and solid tumors (Gregory, Tursz et al. 1987, Nudelman, Deutsch et al. 1987, Maloney and Lingwood 1994, Farkas-Himsley, Hill et al. 1995, Maloney, Binnington-Boyd et al. 1999, Johansson, Johansson et al. 2006) but it is not present in most normal cells. Staining of Gb3 and Hsp70 on the plasma membrane of Hsp70-positive tumor cells revealed their co-localization. Moreover, cholesterol depletion results in a loss of Hsp70 from the plasma membrane of tumor cells (Gehrmann, Liebisch et al. 2008). Previous work by Lingwood et al. has demonstrated that Hsp70 also binds to 3`-sulfogalactolipids via its ATPase domain (NBD) (Fig. 1A) (Mamelak and Lingwood 2001). Based on binding patterns of antibodies that detect different epitopes of Hsp70 in the ATPase and the C-terminal substrate binding domain, the orientation of Hsp70 in Gb3 containing membrane domains

appears to support the above result. Together with the finding that recombinant Hsp70 specifically interacts with artificial lipid vesicles containing Gb3, this supports the hypothesis that Gb3 might be one of the tumor-enriched lipid components that enables the integration of Hsp70 in the plasma membrane of tumor cells (Gehrmann, Liebisch et al. 2008).

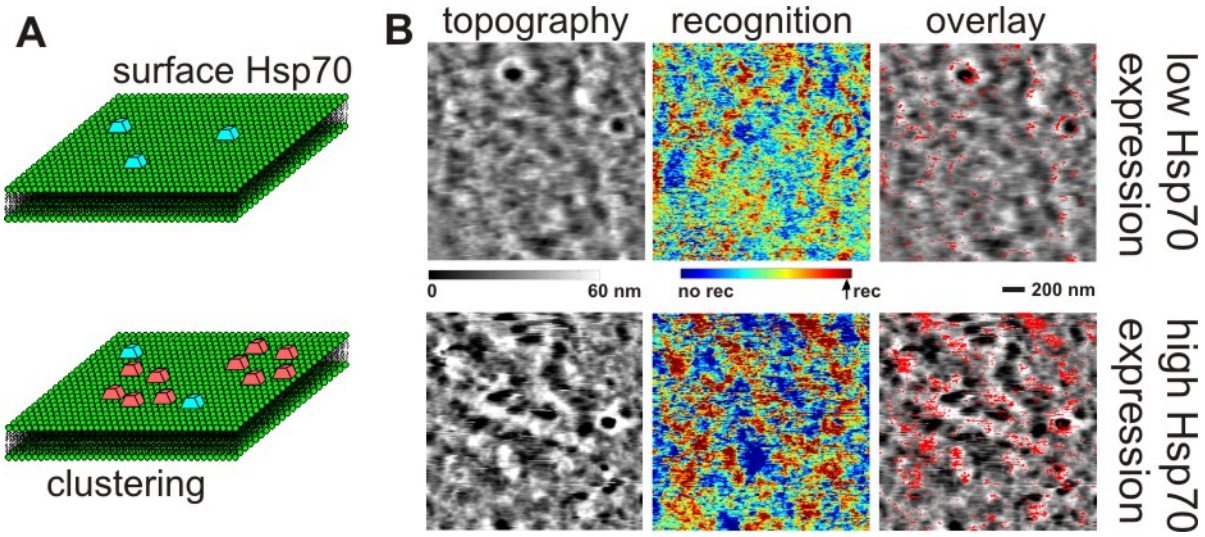


**Fig.4 Hsp70 clustering at the tumor cell surface** Hsp70 forms larger size of nano-domains in the cell membrane of tumor cells expressing higher level of intracellular Hsp70. **(A)** Model of plasma membrane-bound Hsp70, where blue and red symbols correspond to basal (low level) and excess Hsp70, respectively. **(B)** Topography, atomic force microscopy recognition and overlay images. Note that only red pixels above the recognition threshold (rec) are shown in overlay images. These areas are found Hsp70 positive (Nimmervoll, Chtcheglova et al. 2015). Data are displayed with courtesy of Dr. Lilia Chtcheglova and Prof. Peter Hinterdorfer, Johannes Kepler Univertity, Linz, Austria. For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

Apart from the glycosphingolipid Gb3, Hsp70 has been found to interact with artificial lipid bilayers in the presence of phosphatidylserine (PS) (Arispe, Doh et al. 2002, Arispe, Doh et al. 2004). The group of DeMaio has shown that the interaction of Hsp70 with PS is largely based on the negative charge of phospholipids (Armijo, Okerblom et al. 2014). PS residing in liposomes enables the insertion of Hsp70 into the lipid bilayer and thereby can form higher molecular weight oligomers that facilitate ion conductance in artificial lipid

bilayers (Lopez, Cauvi et al. 2016). Assuming that PS serves as the natural binding partner for Hsp70 *in vivo*, a higher PS content would be expected in Hsp70 membrane-positive tumor sublines. In non-stressed cells, PS is predominantly found on the inner membrane layer, whereas, upon stress PS can switch to the outer membrane leaflet, where it can be determined by a specific cell surface staining using the $Ca^{2+}$-dependent phospholipid binding protein Annexin A5. PS on the outer membrane leaflet is considered as an early marker for apoptotic cell death in many cell types where it acts as an "eat-me" signal for macrophages (van den Eijnde, Boshart et al. 1998). However, in the case of tumor cells PS can also be present on the outer membrane leaflet of viable, therapy-resistant, hypoxic cells (Schilling, Gehrmann et al. 2009). It appears that under non-stressed conditions, Hsp70 predominantly resides in membrane clusters whereas following stress Hsp70 often co-localizes with PS outside these clusters. In line with this, atomic force microscopy combined with antigen specific recognition of surface Hsp70 demonstrated that plasma membrane bound Hsp70 forms large clusters and rings potentially surrounding endocytic sites in the cell membrane at higher intracellular and cell surface Hsp70 concentrations (Fig. 4). Shown in both the cell membrane and reconstituted systems clustering was found to depend on the ability of Hsp70 to oligomerize, and larger nano-domains (above 70 nm in diameter) of surface Hsp70 correlated with its ability to facilitate endocytosis in cancer cells (Nimmervoll, Chtcheglova et al. 2015, Chtcheglova and Hinterdorfer 2018).

## 6. Immunological role and therapeutic exploitation of membrane-bound and extracellular HSP70

Significant amounts of membrane-associated Hsp70 are often indicative of highly aggressive tumors, metastatic potential and resistance to therapy (Multhoff, Botzler et al. 1997, Ciocca and Calderwood 2005, Murakami, Kuhnel et al. 2015). However, Hsps with molecular weights ranging from 70 to 90 kDa also elicit protective anti-tumor immune responses if expressed on the plasma membrane or in the extracellular milieu. Previous work of Multhoff and colleagues reported that in the presence of interleukin-2 (IL-2), plasma membrane-bound Hsp70 acts as a tumor-specific recognition structure for natural killer (NK) cells pre-activated with Hsp70 protein (Multhoff, Botzler et al. 1997, Multhoff,

Botzler et al. 1998, Multhoff, Mizzen et al. 1999) or a peptide derived thereof (TKD) (Multhoff, Pfister et al. 2001). In contrast, resting NK cells of tumor patients are unable to kill Hsp70 membrane-positive tumor cells. Since the induction of the cytolytic activity of NK cells with TKD/IL-2 is dose-dependent and saturable, it has been assumed that the stimulation of NK cells with Hsp70 peptide might be mediated via receptors. Blocking experiments revealed that the C-type lectin receptor CD94 in combination with the activatory co-receptor NKG2C as well as other activatory receptors such as the homodimeric receptor NKG2D and natural killer receptors (NKp30, NKp44, NKp46, NKp80) can act as mediators of the interaction of NK cells with Hsp70 membrane-positive tumor cells (Borrego, Masilamani et al. 2006, Sullivan, Clements et al. 2007, Biassoni 2009, Hromadnikova, Li et al. 2016). Following binding of these NK cell receptors to membrane-bound Hsp70, the production and release of the serine protease granzyme B and perforin is initiated which, in turn, results in apoptotic cell death of the tumor cell (Gross, Hansch et al. 2003, Gastpar, Gehrmann et al. 2005). Even in the absence of perforin, granzyme B has been found to interact with membrane-bound Hsp70 on tumor cells. Following binding and uptake of granzyme B into tumor cells via Hsp70-mediated endocytosis, apoptosis can thus be induced (Gehrmann, Stangl et al. 2012). It remains a matter of debate how granzyme B induces tumor cell apoptosis after endo-lysosomal transfer via an Hsp70 pathway.

Depending on the Hsp profile of the lipid surface of actively released exosomes derived from tumor cells (Gastpar, Gehrmann et al. 2005, Lv, Wan et al. 2012) either stimulatory or inhibitory NK-mediated immune responses can be elicited. In the presence of immunogenic peptides that are chaperoned by extracellular Hsps also adaptive immune responses can be initiated following peptide cross-presentation via antigen presenting cells (Udono and Srivastava 1993, Srivastava 2002). Another mechanism whereby extracellular Hsp70 might be able to stimulate tumor cell death is the complex formation of the innate immunity protein Tag7 with Hsp70. It has been shown that the interaction of the Tag7-Hsp70 complex with TNFR1 triggers the activation of RIP1-kinase, an increase in intracellular concentration of $Ca^{2+}$ and an activation of calpains, a family of $Ca^{2+}$ dependent cytoplasmic cysteine proteases, which result in the permeabilization of lysosomal membranes (Yashin, Romanova et al. 2016). The lysosome-induced release

of cathepsins B and D can depolarize mitochondrial membranes and induce ROS production which eventually initiates tumor cell necroptosis (Yashin, Romanova et al. 2016). In contrast to tumor cells, Hsp70 which is released by normal human monocytes in response to granulocyte monocyte-colony stimulating factor (GM-CSF) can prevent the formation of gap-junction intercellular communication between capillary cells and monocytes, and thus could affect inflammation and tumor growth (Thuringer, Berthenet et al. 2015). An anti-inflammatory cardioprotective effect could be shown by plasma exosomes expressing CD63, CD81 and Hsp70 derived from healthy donors (Vicencio, Yellon et al. 2015). This protective effect has been found to be dependent on Hsp70/Toll-like receptor 4 (TLR4) interactions and an activation of kinases that stimulate Hsp27. In summary, depending on the source of the releasing cell type (tumor or normal cells) and the micromilieu (e.g. hypoxia (Rankin and Giaccia 2016)) Hsp-bearing exosomes can exert contradictory immunological responses.

Patients with highly aggressive tumors have elevated levels of serum exosomes, which regulate cell-cell communication by transferring molecules such as cytosolic proteins (including Hsps), lipids, microRNAs and mRNAs (Peinado, Lavotshkin et al. 2011). Hsp70 membrane-positive tumor cells secrete exosomes carrying Hsp70 on their membranes (Gastpar, Gehrmann et al. 2005). Extracellular as well as membrane-bound Hsp70 fulfil dual functions of mediating therapy resistance (Murakami, Kuhnel et al. 2015) and playing pivotal roles in anti-tumor immune responses (Multhoff, Botzler et al. 1997). Hsp70 membrane-positive tumor cells have been found to be significantly more susceptible to the lysis of Hsp70-peptide and IL-2 activated NK cells as compared to their Hsp70 membrane-negative counterparts (Multhoff, Botzler et al. 1995, Multhoff, Botzler et al. 1997). At present the capacity of *ex vivo* TKD/IL-2-stimulated NK cells to kill autologous tumor cells is being tested in a clinical phase II trial in patients with non-small cell lung cancer after radiochemotherapy (Gunther, Ostheimer et al. 2015, Specht, Ahrens et al. 2015).

Furthermore, surface Hsp70 positive exosomes derived from tumor cells have been found to stimulate the migratory and cytolytic capacity of NK cells (Gastpar, Gehrmann et al. 2005). In line with this finding, an intratumoral injection of recombinant Hsp70 into patients with glioblastoma has been shown to induce an increased cytolytic activity of NK cells

and a cytokine shift towards a T helper 1 (Th1)-mediated immune response in preclinical models (Shevtsov, Pozdnyakov et al. 2014) and a pilot study in human patients (Shevtsov, Komarova et al. 2014). Apart from recombinant Hsp70 protein that interacts with membrane Hsp70 through its oligomerization domain (Daugaard, Rohde et al. 2007), the serine protease granzyme B has been found to interact with membrane Hsp70 on tumor cells. Following binding and Hsp70-mediated recycling endosomes, granzyme B induces tumor-specific apoptosis via perforin-independent pathway (Gehrmann, Stangl et al. 2012). Regarding these findings EGFR targeting granzyme B which is overexpressed in NK cells has been found to enhance tumor apoptosis (Oberoi, Jabulowsky et al. 2013). The presence of perforin oligomers induces a rapid plasma membrane flip-flop of phospholipids that facilitate the translocation of granzyme B across plasma membrane bilayers (Metkar, Wang et al. 2011). HS-1 associated protein X-1 (HAX-1), a protein that is involved in the maintenance of the mitochondrial membrane potential also serves as a target for granzyme B. After granzyme B-mediated HAX-1 cleavage, the N-terminal part stimulates mitochondrial depolarization and subsequent lysosomal degradation (Chi, Zhu et al. 2010).

## 7. HSP70 as a regulator of lysosomal lipid catabolism and membrane stability

As discussed above, ample amounts of Hsp70 are found on the surface of cancer cells (Multhoff, Botzler et al. 1997, Hantschel, Pfister et al. 2000). Since high endocytic activity being a characteristic of cancer cells, it is, therefore, not surprising that their lysosomal membranes also contain this protein (Nylandsted, Jäättelä et al. 2004, Mambula and Calderwood 2006). More surprisingly and contrary to most other proteins ending up in the lysosomal lumen, Hsp70 is capable of resisting lysosomal hydrolases and of remaining functional in this hostile environment (Nylandsted, Gyrd-Hansen et al. 2004, Kirkegaard, Roth et al. 2010). The resistance to hydrolysis is likely due to the effective, pH-dependent anchorage of Hsp70 to the lysosomal membranes via its high-affinity binding to bis(monoacylglycero)phosphate (BMP, lysobisphosphatidic acid), an anionic phospholipid abundant in lysosomes (Kirkegaard, Roth et al. 2010). BMP accumulates predominantly in the membranes of intraluminal vesicles (ILV) of the endolysosomal system, and is critical for the formation of ILVs (Matsuo, Chevallier et al. 2004).

Fluorescence spectroscopy-based analyses of BMP-Hsp70 interactions suggest that BMP attaches to both the ATP- and the substrate-binding domain of Hsp70 in an extended conformation with acyl chains inserting into hydrophobic crevices within Hsp70 (Mahalka, Kirkegaard et al. 2014). This anchorage is expected to cause a stringent orientation of Hsp70 on the membrane surface and to induce a transition of its substrate-binding domain into an intermediate conformational state, which may be essential to retain substrate interactions within the hydrophobic bilayer interior. The functionality of lysosomal Hsp70 is supported by accumulating data showing that not only Hsp70 expressed in cells, but also extracellularily added recombinant Hsp70 taken up by endocytosis and accumulating in lysosomes, regulates lysosomal lipid catabolism and lysosomal membrane integrity (Jäättelä, Wissing et al. 1998, Nylandsted, Jäättelä et al. 2004, Hwang, Ryu et al. 2005, Gyrd-Hansen, Farkas et al. 2006, Bivik, Rosdahl et al. 2007, Doulias, Kotoglou et al. 2007, Kirkegaard, Roth et al. 2010, Rammer, Groth-Pedersen et al. 2010, Mena, Rodriguez et al. 2012, Ellegaard, Groth-Pedersen et al. 2013, Zhu, Yoshimoto et al. 2014, Kirkegaard, Gray et al. 2016). As discussed below, these cytoprotective, lysosomal functions of Hsp70 open new possibilities to inhibit and promote cell death in the treatment of various degenerative diseases and cancer, respectively.

## 8. Lysosomes and lysosome-related disorders

Lysosomes are cytosolic vesicles that function as cellular recycling stations, where over 50 acid hydrolases digest all major macromolecules of the cell to breakdown products available for metabolic reutilization (Saftig and Klumperman 2009). Additionally, they serve as major endocytic, $Ca^{2+}$ signaling and more recently as metabolic hubs that sense the nutrient availability and translate it to appropriate signaling pathways (Lloyd-Evans, Morgan et al. 2008, Lloyd-Evans, Waller-Evans et al. 2010, Settembre, Fraldi et al. 2013, Bar-Peled and Sabatini 2014). Lysosomal membranes can be divided into the limiting membrane and any internal membranes of ILVs (Kolter and Sandhoff 2009). These differ significantly in their function and composition. The internal membranes are the sites of lipid degradation. As previously mentioned, they are characterized by high levels of an anionic phospholipid, BMP, whose negative charge serves as a docking site for positively

charged domains of lysosomal lipases (e.g. acid sphingomyelinase) or their cofactors (e.g. saposin) (Kolter and Sandhoff 2009). At the same time, the limiting membrane serves as a barrier that inhibits lethal leakage of lysosomal hydrolases into the cytosol while controlling the proper exchange of ions and the export of metabolites (Saftig and Klumperman 2009). Heavily glycosylated luminal tails of lysosomal-associated membrane proteins (e.g. LAMP-1 and LAMP-2) form a protective glycocalyx shield to the inner face of the membrane (Eskelinen, Tanaka et al. 2003), and numerous channel-forming proteins transport ions and metabolites across the lysosomal membrane (Lloyd-Evans, Waller-Evans et al. 2010, Lloyd-Evans 2016).

Deficiency or malfunction of various lysosomal hydrolases or their co-factors, transport proteins or membrane proteins leads to chronic, often lethal, lysosomal storage disorders that affect many organs, most critically brain (Futerman and van Meer 2004, Ballabio and Gieselmann 2009). In addition to the classic lysosomal storage disorders, that are the most common cause of childhood neurodegeneration (Lloyd-Evans and Haslett 2016), milder lysosomal dysfunction may contribute to pathologies of more common human diseases, such as neurodegeneration (Bourdenx and Dehay 2016, Lloyd-Evans and Haslett 2016, Stoka, Turk et al. 2016). Moreover, lysosomal hyper-activation has recently emerged as a hallmark of metastatic cancer (Kallunki, Olsen et al. 2013, Olson and Joyce 2015, Hämälistö and Jäättelä 2016). Although lysosomal storage disorders can be of mutation origin in over 50 different lysosomal or lysosome-regulating genes, also the accumulation of storage material and the resulting dysfunction of lysosomes results in overlapping tissue pathology and clinical symptoms, with cell death and neuronal loss being marked features in critically ill patients. Loss of lysosomal membrane integrity and release of lysosomal hydrolases to the cytosol can be acutely lethal to cells. As the primary point of no return in a wide variety of cell death cascades (Boya and Kroemer 2008, Kirkegaard and Jäättelä 2009, Aits and Jäättelä 2013, Appelqvist, Waster et al. 2013), lysosomal leakage may, in turn, cause cellular and organ dysfunction developed during chronic lysosomal dysfunction. This view is supported by the demise of cells observed in samples from patients with some lysosomal storage disorders (Kirkegaard, Roth et al. 2010, Kollmann, Damme et al. 2012, Kollmann, Uusi-Rauva et al. 2013, Micsenyi, Sikora et al. 2013), and cancer cell death following lysosome-targeting

therapies (reviewed in (Kirkegaard and Jäättelä 2009, Groth-Pedersen and Jäättelä 2013)). Notably, cancer cells either overexpressing Hsp70 or treated with recombinant Hsp70 are significantly protected against lysosomal leakage and subsequent cell death, whereas those depleted of Hsp70 undergo spontaneous lysosomal membrane permeabilization, or become more susceptible to lysosome-disruptive stimuli (Jäättelä, Wissing et al. 1992, Jäättelä, Wissing et al. 1998, Nylandsted, Jäättelä et al. 2004, Hwang, Ryu et al. 2005, Gyrd-Hansen, Farkas et al. 2006, Bivik, Rosdahl et al. 2007, Doulias, Kotoglou et al. 2007, Kirkegaard, Roth et al. 2010, Rammer, Groth-Pedersen et al. 2010, Mena, Rodriguez et al. 2012, Ellegaard, Groth-Pedersen et al. 2013, Petersen, Olsen et al. 2013, Subrizi, Toropainen et al. 2015).

## 9. Lysosomal membrane integrity is regulated by HSP70

Maintenance of the lysosomal membrane integrity is of utmost importance for cellular homeostasis and survival. Yet, our knowledge on the mechanisms regulating lysosomal membrane permeability is only beginning to emerge. Among the emerging lysosomal membrane destabilizers are certain lipids and reactive oxygen species (Aits and Jäättelä 2013, Appelqvist, Waster et al. 2013), both of which can be regulated by Hsp70. Sphingomyelin, arachidonic acid and possibly high concentrations of sphingosine promote lysosomal leakage, cell death and enhanced pathology in cells and tissues from lysosomal storage disease patients (Kågedal, Zhao et al. 2001, Feldstein, Werneburg et al. 2004, Zhang, Yi et al. 2006, Kirkegaard, Roth et al. 2010, Ellegaard, Groth-Pedersen et al. 2013, Petersen, Olsen et al. 2013). The ability of Hsp70 to stabilize lysosomal membranes has been largely attributed to its ability to enhance sphingolipid catabolism in the lysosomes through its high-affinity binding to BMP (Kirkegaard, Roth et al. 2010, Petersen, Olsen et al. 2013, Kirkegaard, Gray et al. 2016). As discussed above, this anionic phospholipid is an essential cofactor for lysosomal sphingolipid catabolism (Kolter and Sandhoff 2005). Via its negative charge, it tethers several sphingolipid-degrading enzymes to the internal lysosomal membranes where their substrates are located, thereby increasing their activity and protecting them from lysosomal degradation. The high-affinity association of Hsp70 and BMP, which protects Hsp70 from lysosomal degradation as discussed above, also facilitates the BMP binding of sphingolipid-

degrading enzymes and, in so doing, further enhances their activity and inhibits their degradation (Kirkegaard, Roth et al. 2010, Mahalka, Kirkegaard et al. 2014, Kirkegaard, Gray et al. 2016). The lysosomal membrane-stabilizing effect of Hsp70 may rely in particular on its enhancing effect on the enzyme acid sphingomyelinase that hydrolyses sphingomyelin to ceramide and phosphocholine (Fig. 5). Hsp70-induced conversion of ILV-sphingomyelin to ceramide counteracts lysosomal aggregation and membrane permeabilization, which are hallmarks of stress-induced cell death and may contribute to cellular pathophysiology in some lysosomal storage disorders (Kirkegaard, Roth et al. 2010, Micsenyi, Sikora et al. 2013, Petersen, Olsen et al. 2013, Te Vruchte, Speak et al. 2014). The mechanism by which accumulating ceramide stabilizes lysosomes remains largely unknown. Nevertheless, level of very long chain ceramide species ($C_{24:0}$, $C_{24:1}$, $C_{24:2}$) was significantly increased in Hsp70 transgenic mouse embryonic fibroblast (MEF) cells as compared to their controls (Kirkegaard, Roth et al. 2010). While short to long chain ceramides are frequently considered as mediators of cellular death, very long chain ceramide species may protect membrane integrity and confer survival benefit on cells (Hartmann, Lucks et al. 2012, Stiban and Perera 2015, Rudd and Devaraj 2018). It is likely that ceramides accumulated in the lysosome eventually influence other cellular membranes, therefore affecting lysosomal stability indirectly as well (van Blitterswijk, van der Luit et al. 2003). If the plasma membrane integrity should be severely impaired lysosomal ASM, facilitated by Hsp70 may be also exposed to the cell surface, where ceramide-enriched platforms seal the membrane. This is achieved by conical shaped ceramides capable of inducing membrane invaginations hence facilitating vesicle budding and fission (Andrews, Almeida et al. 2014). Increased concentration of lysosomal ceramide counteracts aggregation of lysosomes with other intracellular vesicles and membranes, and perhaps strengthen the lysosomal limiting membranes by its ability to shape membranes (Heinrich, Wickel et al. 2000) Interestingly, Hsp70 could enhance also the catabolism of several less abundant sphingolipids (Kirkegaard, Gray et al. 2016), whose role in the maintenance of lysosomal membrane integrity remains to be studied. Of special interest is the enzyme galactosylceramidase, whose loss of activity results in accumulation of galactosylsphingosine (a.k.a. psychosine) that disrupts lysosomal pH (Folts, Scott-Hewitt et al. 2016), possibly destabilizing the lysosomal membranes by

interaction with the pH sensitive ion channel TDAG8 (Wang, Kon et al. 2004). Finally, it should be also noted that Hsp70-facilitated sphingomyelin degradation and concomitant ceramide accumulation allows a Niemann-Pick C2 (NPC2) mediated cholesterol egress from the lysosome (Infante, Wang et al. 2008, Oninla, Breiden et al. 2014), which is expected to affect membrane integrity and cell survival in multiple ways.

In addition to regulating lysosomal lipid catabolism, Hsp70 may regulate lysosomal membrane stability by protecting the membranes from oxidative stress. Inside the lysosomes, iron and other chemically reactive metals (e.g. copper, zinc and cobalt) can generate reactive oxygen species through Fenton-type chemical reactions, which can lead to oxidization and destabilization of membrane lipids (Kurz, Eaton et al. 2010, Kiselyov, Colletti et al. 2011). Interestingly, one of the common pathologies in various lysosomal storage diseases is the marked elevation of oxidative stress providing a possible mechanistic clue to the loss of lysosomal integrity and cell death occurring in these diseases (Jeyakumar, Thomas et al. 2003, Shen 2008, Zampieri, Mellon et al. 2009, Vitner, Farfel-Becker et al. 2012). Importantly, the well documented protective effect of Hsp70 against oxidative stress is preserved inside the lysosomes. In the case of photo-oxidation of acridine orange-loaded lysosomes, real-time high-resolution imaging has demonstrated that Hsp70 localized in the lysosomal lumen effectively protects lysosomal membranes and thereby mitigates their destabilization upon local oxidative stress (Kirkegaard, Roth et al. 2010). Furthermore, Hsp70 is cytoprotective in other lysosomal oxidative stress models, including age-related macular degeneration of retinal pigment epithelium and lysosomal iron accumulation (Nylandsted, Gyrd-Hansen et al. 2004, Doulias, Kotoglou et al. 2007, Subrizi, Toropainen et al. 2015). It remains to be studied, whether Hsp70 has a direct antioxidant effect or whether the protection of lysosomal membranes against oxidative stress is due to indirect effects, such as changes in the lipid composition of the membranes.

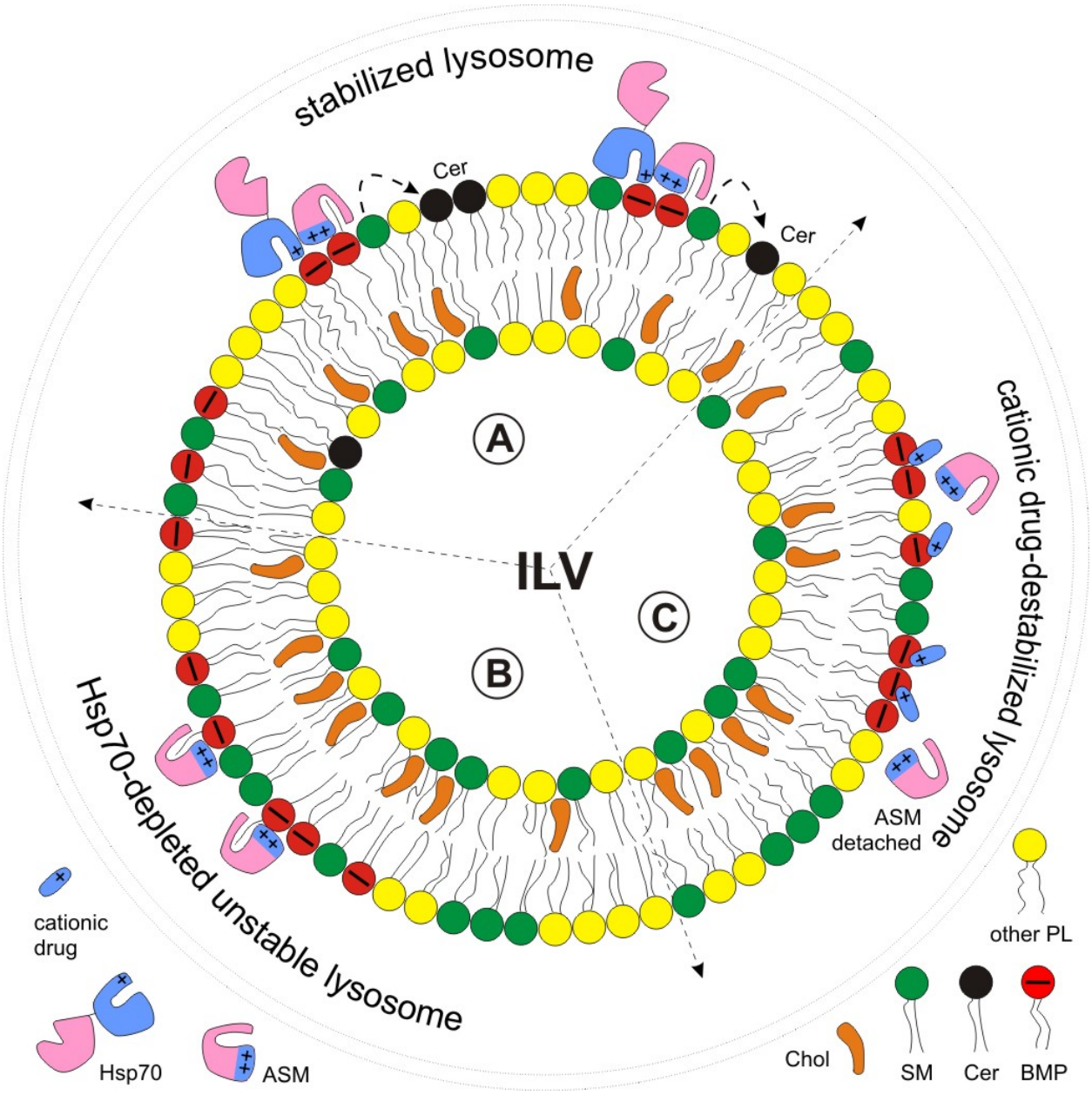


**Fig.5 Hsp70-mediated preservation of lysosomal membrane integrity** Lysosomal membranes, in particular those of intraluminal vesicles (ILVs) are enriched in bis(monoacyl)glycerophosphate (BMP) of inverted conical shape that allows high curvature membrane formation. (A) Negative charge of the head group of BMP recruits acid sphingomyelinase (ASM) as well as Hsp70 to the membrane surface. ASM converts sphingomyelin (SM) to membrane stabilizing ceramide (Cer), which is largely dependent on Hsp70 bound to the luminal side of ILVs. Hsp70 dependent activation of ASM and other lysosomal lipases eventually changes the lipid composition of all cellular membranes. (B) Hsp70 depletion generates lysosome instability, triggering in turn lysosomal membrane permeabilization (LMP)-

mediated cell death. (C) Alternatively, cationic lysosomotropic drugs neutralize the negative charge of bis(monoacyl)glycerophosphate (BMP), that ASM and Hsp70 are anchored to, therefore causing lysosomal instability, LMP and cell death. PL: glycerophospholipid, Chol: cholesterol, double circle: limiting membrane

## 10. Therapeutic exploitation of lysosomal HSP70 function

After the initial discoveries of the role of Hsp70 in lysosomal membrane stability and lysosomal lipid catabolism (Nylandsted, Gyrd-Hansen et al. 2004, Kirkegaard, Roth et al. 2010), a number of recent publications have reported improved lysosomal enzyme activities and lysosomal function through the induction of heat shock proteins in various lysosomal storage disorders (Mu, Ong et al. 2008, O'Leary and Igdoura 2012, Nakasone, Nakamura et al. 2014, Yang, Swallows et al. 2014, Zhu, Yoshimoto et al. 2014, Kirkegaard, Gray et al. 2016). As a consequence, the induction of the heat shock response is emerging as an attractive therapeutic approach to treat these devastating diseases. The power of this approach is supported by recent data showing that recombinant Hsp70 can reverse lysosomal pathology in primary fibroblasts from eight different lysosomal storage disorders and has significant therapeutic effects on both substrate accumulation and neurological manifestations in murine models of three of them, i.e. Fabry, Sandhoff and Niemann-Pick type C diseases (Kirkegaard, Gray et al. 2016). Notably, these therapeutic effects of recombinant Hsp70 can be recapitulated by oral administration of arimoclomol, a small molecule co-inducer of heat shock proteins, currently in clinical trials for Niemann-Pick disease type C (Kirkegaard, Gray et al. 2016). It should be noted that the therapeutic effects of Hsp70 in lysosomal storage diseases are not confined to its direct effects in lysosomes, but are likely to depend also on the classic chaperone functions of Hsp70.

Contrary to lysosomal storage disorders and degenerative disease, where increased lysosomal Hsp70 activity appears to have a beneficial effect, the inhibition of lysosomal Hsp70 function is emerging as an attractive approach to treat cancer. Whereas the direct inhibition of Hsp70 in the lysosomal lumen may be technically challenging, the inhibition of its target, acid sphingomyelinase, can be easily achieved. In fact, over a hundred commonly used, FDA-approved drugs are functional inhibitors of this enzyme (Kornhuber, Tripal et al. 2010). These drugs are characterized by a hydrophobic ring structure and a

hydrophilic side chain with a cationic amine group. In the acidic pH of lysosomes, the basic amine groups are protonated resulting in an up to 1000-fold accumulation (Trapp, Rosania et al. 2008). The incorporation of such cationic amphiphilic drugs into membranes in the lysosomal lumen neutralizes the negative membrane charge and inhibits the function of several lysosomal lipases, including acid sphingomyelinase (Kolzer, Werth et al. 2004). Thus, they have exactly the opposite effect to lysosomal Hsp70 (Fig. 5). Importantly, cancer cells are especially sensitive to the accumulation of sphingomyelin (Barcelo-Coblijn, Martin et al. 2011, Teres, Llado et al. 2012, Petersen, Olsen et al. 2013), which may explain why these functional inhibitors of acid sphingomyelinase display selective cytotoxicity towards transformed cells both *in vitro* and in various cancer models in mice (Groth-Pedersen, Ostenfeld et al. 2007, Ostenfeld, Høyer-Hansen et al. 2008, Jahchan, Dudley et al. 2013, Petersen, Olsen et al. 2013, Sukhai, Prabha et al. 2013, Shchors, Massaras et al. 2015, Ellegaard, Dehlendorff et al. 2016). Their putative efficacy in cancer treatment is further supported by a recent pharmaco-epidemiological register-based cohort study showing a statistically significant association between cationic amphiphilic antihistamine use and reduced mortality among Danish cancer patients (Ellegaard, Dehlendorff et al. 2016).

## 11. HSP70 disorder and lysosomal-mediated neuronal death

Lysosome-dependent cell death is characterized by the destabilization of its limiting membrane (Fig. 5) followed by the leakage of cathepsins from the lysosomal lumen into the cytoplasm (Brunk, Zhang et al. 1995, Brunk, Dalen et al. 1997, Brunk and Svensson 1999, Brunk, Neuzil et al. 2001, Aits and Jäättelä 2013, Lipton 2013, Gomez-Sintes, Ledesma et al. 2016). Using the monkey experimental systems of transient brain ischemia, Yamashima et al. (Yamashima, Saido et al. 1996, Yamashima, Kohda et al. 1998, Yamashima 2000) formulated the ‘calpain-cathepsin hypothesis’ as a mechanism of programmed neuronal necrosis. They demonstrated that the lysosomal membrane of hippocampal CA1 neurons is disrupted by the activated μ-calpain after transient ischemia, which causes the release of lysosomal cathepsins B and L. Thereafter, the ‘calpain-cathepsin hypothesis’ has been confirmed, using a variety of experimental paradigms from *C. elegans* to rodents (Syntichaki, Xu et al. 2002, Ceccariglia, D'Altocolle et al. 2011,

Villalpando Rodriguez and Torriglia 2013, Koriyama, Sugitani et al. 2014). The role of lysosomal enzyme cathepsins in initiation and execution of the necrotic cell death program has become clear (Boya and Kroemer 2008, Aits and Jäättelä 2013, Zhu, Yoshimoto et al. 2014). Moreover, the brain and neurons are regularly exposed to different kinds of acute and chronic environmental stresses. The brain contains high levels of polyunsaturated fatty acids and redox transition metal ions, especially iron. In spite of its high oxygen consumption, however, levels of lower molecular weight and enzymatic antioxidants are relatively low in the brain. Accordingly, the brain with poor antioxidant defense appears particularly susceptible to lipid peroxidation by reactive oxygen species (Chong, Li et al. 2005). Peroxidation of membrane lipids may show numerous effects such as increased membrane rigidity, decreased membrane-bound enzyme activity, altered membrane receptor activity, and altered membrane permeability. Therefore, it is not surprising that the role of lipid peroxidation has been widely investigated in the pathogenesis of a variety of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, Down syndrome (Perluigi, Coccia et al. 2012).

Importantly, lipid peroxidation yields a variety of bioactive products and one of the most extensively studied examples is hydroxynonenal (Dalleau, Baradat et al. 2013, Schaur, Siems et al. 2015). The most common source of hydroxynonenal is an endogenous one, when it is produced by peroxidation of membrane phospholipids or plasma low-density lipoproteins. Hydroxynonenal generation in the brain has been associated with exposure to drugs, ethanol or irradiation, and with ischemia or inflammation. In contrast, exogenous hydroxynonenal is generated during food processing, i.e., heating, especially deep-frying, of ω-6 vegetable oils (Fig. 6 A,B) (Dalleau, Baradat et al. 2013). Because of its chemical reactivity, hydroxynonenal can exert pleiotropic effects notably cell death. After the ischemia/ reperfusion sequence in myocardial infarction, accumulated reactive oxygen species promote generation of hydroxynonenal, which disrupts actin cytoskeleton, alters $Ca^{2+}$ homeostasis, and triggers cardiomyocyte cell death (VanWinkle, Snuggs et al. 1994). Hydroxynonenal induces signaling for apoptosis via both the Fas-mediated extrinsic and the p53-mediated intrinsic pathways (Chaudhary, Sharma et al. 2010, Dalleau, Baradat et al. 2013). Thus, hydroxynonenal can trigger β- cell apoptosis in the

pancreas, and induce glucose intolerance and type 2 diabetes (Mattson 2009). Since hydroxynonenal can impair $Na^{+}/Ca^{2+}$ pumps and glucose and glutamate transporters by modifying membranes, the resultant ionic and energetic disturbances cause neuronal cell death (Keller, Pang et al. 1997, Mark, Lovell et al. 1997). However, the detailed mechanism of how hydroxynonenal can lead to cell death has been controversial until recently.

Accumulated data suggest dual roles of Hsp70 not only as a molecular chaperone for altered (misfolded/ aged/ damaged) proteins but also as a guardian of lysosomal integrity (Kirkegaard, Roth et al. 2010, Petersen and Kirkegaard 2010, Yamashima 2012, Yamashima 2013). Hsp70 contributes to lysosomal stabilization (Fig. 5) by binding to the endolysosomal anionic phospholipid BMP, a co-factor essential for sphingomyelin catabolism (Kirkegaard, Roth et al. 2010). Membranes of ILVs of the functioning lysosomes are characterized by abundant BMP (Kolter and Sandhoff 2009, Schulze, Kolter et al. 2009). Then, Hsp70-BMP binding enhances activity of acid sphingomyelinase, which mediates the sphingolipid degradation at the internal membrane in the acidic (pH4.5) compartment to generate ceramide (Linke, Wilkening et al. 2001, Linke, Wilkening et al. 2001, Kolter and Sandhoff 2005). Ceramide protects lysosomal membrane integrity as discussed above (Kirkegaard, Roth et al. 2010, Petersen and Kirkegaard 2010, Petersen, Kirkegaard et al. 2010) (Fig. 5A). Thus, in cases of Hsp70 depletion, not only failure of protein trafficking and degradation but also lysosomal destabilization or rupture may occur (Fig. 5B). In the monkey hippocampal CA1 neurons after transient ischemia, Oikawa et al. (Oikawa, Yamada et al. 2009) previously found by proteomic analysis that Hsp70 can become an *in vivo* target of carbonylation by hydroxynonenal (Fig. 6C). Intriguingly, carbonylation of Hsp70 increased more than ten-fold in the post-ischemic CA1 neurons, compared to the non-ischemic controls. Subsequently, in the *in vitro* experiments, Hsp70 being carbonylated by hydroxynonenal was found to become susceptible to cleavage by activated μ-calpain (Fig. 6D) (Sahara and Yamashima 2010). ‘Calpain-mediated cleavage of carbonylated Hsp70’ can lead to both autophagy failure and lysosomal destabilization with the resultant release of cathepsins and neuronal death (Yamashima and Oikawa 2009).

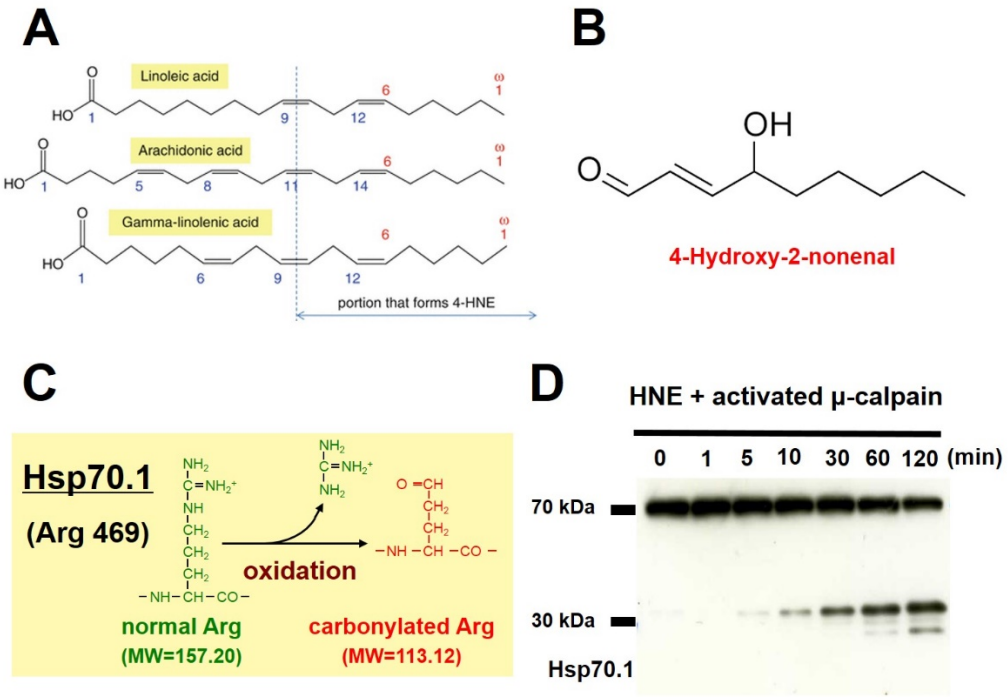


**Fig.6 Generation of hydroxynonenal (HNE), HNE-mediated carbonylation and calpain-mediated cleavage of carbonylated Hsp70** (**A, B**) Generation of hydroxynonenal (4-hydroxy-2-nonenal) from ω-6 polyunsaturated fatty acids such as linoleic, arachidonic and gamma-linolenic acids. (**C**) Carbonylation of Hsp70 occurs at the key site Arg469 in post-ischemic CA1 neurons. (**D**) Time-dependent μ-calpain-mediated cleavage of carbonylated-Hsp70 by hydroxynonenal (HNE) in monkey CA1 tissue. For further details see text.

Although neuronal death in Alzheimer's disease has been thought to be caused by the initial cerebral accumulation of amyloid β for half a century, it still remains enigmatic because the underlying mechanism of Alzheimer neuronal death due to amyloid β still remains unknown. Thus, research of this disease is moving away from the simple assumption of linear causality as proposed in the 'amyloid hypothesis'. Recently, hydroxynonenal has been shown to be involved in a great number of pathologies such as neurodegenerative diseases, metabolic diseases, and cancers (Mattson 2009). Yamashima recently put forward a perspective view that the causative substance for Alzheimer neuronal death is actually ω-6 vegetable oil-derived hydroxynonenal (Yamashima 2016).

Hsp70 is known to recycle altered proteins, stabilize lysosomal membranes and protect cells from diverse oxidative stresses. 'Hydroxynonenal-induced Hsp70 carbonylation' (Fig. 6C) followed by 'calpain-mediated cleavage of carbonylated Hsp70' (Fig. 6D) may be crucial for the execution of both ischemic and degenerative neuronal death. Calpain activation and Hsp70 disorder, combined together, at the lysosomal membranes may bring about programmed neuronal death by releasing hydrolytic cathepsin enzymes (Yamashima, Saido et al. 1996, Yamashima, Kohda et al. 1998, Yamashima 2000). The pathway of cerebral ischemia and/or oxidative stresses, either acute (in case of stroke) or chronic (in case of degeneration) could result the following sequence of μ-calpain activation → excessive intake of ω-6 vegetable oils → increase of hydroxynonenal in the brain → hydroxynonenal-mediated Hsp70 carbonylation → activated μ-calpain-mediated cleavage of carbonylated Hsp70 → lysosomal membrane destabilization → cathepsin release → breakdown of the cell constitutive proteins, which may in turn represent a central role not only for ischemic neuronal death (Yamashima 2000, Yamashima and Oikawa 2009, Yamashima 2012) but also for Alzheimer neuronal death (Yamashima 2013, Yamashima 2016).

A continuum of abnormalities of the lysosomal system can be identified in ischemic and Alzheimer neurons (Nixon, Wegiel et al. 2005, Lloyd-Evans and Haslett 2016). The common characteristic is that functional Hsp70 is indispensable for lysosomal autophagy that is crucial for the homeostasis of neurons. The control of protein turnover is particularly important in post-mitotic cells such as neurons, where accumulation of altered proteins may be highly detrimental to cell survival (Kopito 2000). Neurons must maintain large volumes of membrane and cytoplasm, and continually traffic autophagy-related garbage long distances from distal ends of dendrites and axons back to the cell body where lysosomes are most active for catabolite clearance (Lee, Sato et al. 2011). Hsp70 is the most structurally and functionally conserved chaperone that plays a principal role in the trafficking and degradation of altered proteins and their quality control for the cytoprotection of neurons under a number of different conditions (Yamashima 2016). Accordingly, in case of Hsp70 dysfunction, failure of lysosomal autophagy may occur, which leads to accumulation of autophagic vacuoles in both ischemic and Alzheimer neurons (Nixon, Wegiel et al. 2005). Since the proteotoxic stress in ischemia/reperfusion

during stroke is severe, neurons die within hours or days after the insult. On the contrary, the proteotoxic stress in Alzheimer's diseases is extremely mild, and neurons can battle it for months or years, perhaps by raising pro-survival defenses such as Hsp70. Previous studies (Butterfield, Boyd-Kimball et al. 2003, Butterfield, Abdul et al. 2006, Butterfield, Reed et al. 2006, Butterfield, Reed et al. 2007, Sultana, Perluigi et al. 2010) indicated increased levels of protein oxidation in the Alzheimer brains, and suggested a possible involvement of hydroxynonenal-mediated protein carbonylation for the progression of Alzheimer's disease. When sub-threshold levels of Hsp70 carbonylation are coupled with sub-threshold levels of calpain activation, for example, due to long-standing mild cerebral ischemia and/or amyloid β accumulation, programmed neuronal death becomes steadily significant year by year. Not only in cerebral ischemia but also in Alzheimer's disease, 'calpain-mediated cleavage of carbonylated Hsp70' may cause lysosomal membrane rupture/destabilization, which leads to the release of cathepsins into the cytoplasm, which can then trigger progressive neuronal death. Nowadays, researchers of Alzheimer's disease are gradually but steadily moving away from the classical amyloid hypothesis, and speculate that another age-related, disease-promoting factor and/or substance probably interact with the core mechanisms of the disease. Accordingly, targeting Hsp70 might be a promising strategy for both elucidating the mechanism of Alzheimer neuronal death as well as developing novel therapeutic agents for Alzheimer's disease where defects in lysosomal proteolysis and lipid accumulation have been observed (Lee, McBrayer et al. 2015).

## 12. Concluding remarks

It is now widely accepted that some of the heat shock protein molecular chaperones have additional biological functions over their basic role in cellular proteostasis, i.e. acting as 'moonlighting proteins'. The moonlighting Hsp70 has been emerging an important therapeutic target. However, efforts targeting essential chaperone activity or the interacting complexes of Hsp70 with proteins have not yet resulted in excellent specific and efficient drug candidates of low toxicity (Lazarev, Sverchinsky et al. 2018, Taylor, Dunyak et al. 2018, Yaglom, Wang et al. 2018). Drug design is hampered by the facts that Hsps are highly conserved (Schlecht, Scholz et al. 2013) and that Hsp70, specifically Hsp70.1 has multiple functions. Hsp70 is in fact more than simply a cytosolic chaperone (Horvath, Multhoff et al. 2008, Juhasz, Lipp et al. 2013) and considered a major regulator of signaling pathways (Gabai, Yaglom et al. 2009, Sherman and Gabai 2015, Gabai, Yaglom et al. 2016). As reviewed here, non-cytosolic localization, membrane crossing and lipid interactions of Hsp70 are associated with membrane resistance, facilitation of endocytosis, counteracting apoptotic mechanisms and sustaining survival signaling at pathophysiological states. Unlike roles in the cytosol these unique functions may not only be targeted via Hsp70 itself or its interacting proteins, but also via specific lipids that either interact with or can be modulated by Hsp70. In order for rational drug design of membrane/ lipid-mediated Hsp70 functions, we need further mechanistic and structural studies of Hsp70 membrane interactions and lipid modulation.

**Acknowledgement**

ZB is a Bolyai Research Fellow and supported by UP MS KA-2018-05 and ÚNKP-18-4-PTE-26 new national excellence program of the Ministry of Human Capacities. This work has been supported by also GINOP-2.3.2-15- 2016-00049, GINOP-2.3.3-15-2016-00025 and GINOP-2.3.2-15-2016-00040. Work in the Multhoff laboratory has been supported by DFG (SFB824-3), STA 1520/1-1 BMBF 01GU0823, BMWi (AiF) ZF4320102CS7. Work in the Lloyd-Evans lab is supported by the MRC (MR/P007651/1), BBSRC (BB/S002774/1), EU Horizon 2020 BATcure consortium grant (666918) and Action Medical Research. The related research in the Jäättelä laboratory has been supported by Danish National Research Foundation (DNRF125), European Research Council (AdG 340751), Danish Cancer Society (R167-A11061), Danish Council for Independent Research (DFF-7016-00360), and Novo Nordisk Foundation (NNF15OC0016914).

## References


Adler, V., A. Schaffer, J. Kim, L. Dolan and Z. Ronai (1995). "UV irradiation and heat shock mediate JNK activation via alternate pathways." *J Biol Chem* **270**(44): 26071-26077.

Aits, S. and M. Jäättelä (2013). "Lysosomal cell death at a glance." J *Cell Sci* **126**(Pt 9): 1905-1912.

Andrews, N. W., P. E. Almeida and M. Corrotte (2014). "Damage control: cellular mechanisms of plasma membrane repair." *Trends Cell Biol* **24**(12): 734-742.

Appelqvist, H., P. Waster, K. Kagedal and K. Ollinger (2013). "The lysosome: from waste bag to potential therapeutic target." *J Mol Cell Biol* **5**(4): 214-226.

Aprile, F. A., A. Dhulesia, F. Stengel, C. Roodveldt, J. L. Benesch, P. Tortora, C. V. Robinson, X. Salvatella, C. M. Dobson and N. Cremades (2013). "Hsp70 oligomerization is mediated by an interaction between the interdomain linker and the substrate-binding domain." *PLoS One* **8**(6): e67961.

Arispe, N., M. Doh and A. De Maio (2002). "Lipid interaction differentiates the constitutive and stress-induced heat shock proteins Hsc70 and Hsp70." *Cell Stress Chaperones* **7**(4): 330-338.

Arispe, N., M. Doh, O. Simakova, B. Kurganov and A. De Maio (2004). "Hsc70 and Hsp70 interact with phosphatidylserine on the surface of PC12 cells resulting in a decrease of viability." *FASEB J* **18**(14): 1636-1645.

Armijo, G., J. Okerblom, D. M. Cauvi, V. Lopez, D. E. Schlamadinger, J. Kim, N. Arispe and A. De Maio (2014). "Interaction of heat shock protein 70 with membranes depends on the lipid environment." *Cell Stress Chaperones* **19**(6): 877-886.

Asea, A., G. Ara, B. A. Teicher, M. A. Stevenson and S. K. Calderwood (2001). "Effects of the flavonoid drug quercetin on the response of human prostate tumours to hyperthermia in vitro and in vivo." *Int J Hyperthermia* **17**(4): 347-356.

Asea, A., S. K. Kraeft, E. A. Kurt-Jones, M. A. Stevenson, L. B. Chen, R. W. Finberg, G. C. Koo and S. K. Calderwood (2000). "HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine." *Nat Med* **6**(4): 435-442.

Ballabio, A. and V. Gieselmann (2009). "Lysosomal disorders: from storage to cellular damage." *Biochim Biophys Acta* **1793**(4): 684-696.

Balogh, G., I. Horvath, E. Nagy, Z. Hoyk, S. Benko, O. Bensaude and L. Vigh (2005). "The hyperfluidization of mammalian cell membranes acts as a signal to initiate the heat shock protein response." *FEBS J* **272**(23): 6077-6086.

Balogh, G., G. Maulucci, I. Gombos, I. Horvath, Z. Torok, M. Peter, E. Fodor, T. Pali, S. Benko, T. Parasassi, M. De Spirito, J. L. Harwood and L. Vigh (2011). "Heat stress causes spatially-distinct membrane re-modelling in K562 leukemia cells." *PLoS One* **6**(6): e21182.

Balogh, G., M. Peter, A. Glatz, I. Gombos, Z. Torok, I. Horvath, J. L. Harwood and L. Vigh (2013). "Key role of lipids in heat stress management." *FEBS Lett* **587**(13): 1970-1980.

Balogi, Z., O. Cheregi, K. C. Giese, K. Juhasz, E. Vierling, I. Vass, L. Vigh and I. Horvath (2008). "A mutant small heat shock protein with increased thylakoid association provides an elevated resistance against UV-B damage in synechocystis 6803." *J Biol Chem* **283**(34): 22983-22991.

Balogi, Z., Z. Torok, G. Balogh, K. Josvay, N. Shigapova, E. Vierling, L. Vigh and I. Horvath (2005). ""Heat shock lipid" in cyanobacteria during heat/light-acclimation." *Arch Biochem Biophys* **436**(2): 346-354.

Bar-Peled, L. and D. M. Sabatini (2014). "Regulation of mTORC1 by amino acids." *Trends Cell Biol.*

Barcelo-Coblijn, G., M. L. Martin, R. F. de Almeida, M. A. Noguera-Salva, A. Marcilla-Etxenike, F. Guardiola-Serrano, A. Luth, B. Kleuser, J. E. Halver and P. V. Escriba (2011). "Sphingomyelin and sphingomyelin synthase (SMS) in the malignant transformation of glioma cells and in 2-hydroxyoleic acid therapy." *Proc Natl Acad Sci U S A* **108**(49): 19569-19574.
Barreto, A., J. M. Gonzalez, E. Kabingu, A. Asea and S. Fiorentino (2003). "Stress-induced release of HSC70 from human tumors." *Cell Immunol* **222**(2): 97-104.
Bausero, M. A., R. Gastpar, G. Multhoff and A. Asea (2005). "Alternative mechanism by which IFN-gamma enhances tumor recognition: active release of heat shock protein 72." *J Immunol* **175**(5): 2900-2912.
Beltrao, P., V. Albanese, L. R. Kenner, D. L. Swaney, A. Burlingame, J. Villen, W. A. Lim, J. S. Fraser, J. Frydman and N. J. Krogan (2012). "Systematic functional prioritization of protein posttranslational modifications." *Cell* **150**(2): 413-425.
Biassoni, R. (2009). "Human natural killer receptors, co-receptors, and their ligands." *Curr Protoc Immunol* **Chapter 14**: Unit 14 10.
Bivik, C., I. Rosdahl and K. Ollinger (2007). "Hsp70 protects against UVB induced apoptosis by preventing release of cathepsins and cytochrome c in human melanocytes." *Carcinogenesis* **28**(3): 537-544.
Borrego, F., M. Masilamani, A. I. Marusina, X. Tang and J. E. Coligan (2006). "The CD94/NKG2 family of receptors: from molecules and cells to clinical relevance." *Immunol Res* **35**(3): 263-278.
Bottger, E., G. Multhoff, J. F. Kun and M. Esen (2012). "Plasmodium falciparum-infected erythrocytes induce granzyme B by NK cells through expression of host-Hsp70." *PLoS One* **7**(3): e33774.
Bourdenx, M. and B. Dehay (2016). "What lysosomes actually tell us about Parkinson's disease?" *Ageing Res Rev* **32**: 140-149.
Boya, P. and G. Kroemer (2008). "Lysosomal membrane permeabilization in cell death." *Oncogene* **27**(50): 6434-6451.
Broquet, A. H., G. Thomas, J. Masliah, G. Trugnan and M. Bachelet (2003). "Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release." *J Biol Chem* **278**(24): 21601-21606.
Brown, G., H. W. Rixon, J. Steel, T. P. McDonald, A. R. Pitt, S. Graham and R. J. Sugrue (2005). "Evidence for an association between heat shock protein 70 and the respiratory syncytial virus polymerase complex within lipid-raft membranes during virus infection." *Virology* **338**(1): 69-80.
Brunk, U. T., H. Dalen, K. Roberg and H. B. Hellquist (1997). "Photo-oxidative disruption of lysosomal membranes causes apoptosis of cultured human fibroblasts." *Free Radic Biol Med* **23**(4): 616-626.
Brunk, U. T., J. Neuzil and J. W. Eaton (2001). "Lysosomal involvement in apoptosis." *Redox Rep* **6**(2): 91-97.
Brunk, U. T. and I. Svensson (1999). "Oxidative stress, growth factor starvation and Fas activation may all cause apoptosis through lysosomal leak." *Redox Rep* **4**(1-2): 3-11.
Brunk, U. T., H. Zhang, H. Dalen and K. Ollinger (1995). "Exposure of cells to nonlethal concentrations of hydrogen peroxide induces degeneration-repair mechanisms involving lysosomal destabilization." *Free Radic Biol Med* **19**(6): 813-822.
Butterfield, D. A., H. M. Abdul, S. Newman and T. Reed (2006). "Redox proteomics in some age-related neurodegenerative disorders or models thereof." *NeuroRx* **3**(3): 344-357.
Butterfield, D. A., D. Boyd-Kimball and A. Castegna (2003). "Proteomics in Alzheimer's disease: insights into potential mechanisms of neurodegeneration." *J Neurochem* **86**(6): 1313-1327.

Butterfield, D. A., T. Reed, S. F. Newman and R. Sultana (2007). "Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment." *Free Radic Biol Med* **43**(5): 658-677.
Butterfield, D. A., T. Reed, M. Perluigi, C. De Marco, R. Coccia, C. Cini and R. Sultana (2006). "Elevated protein-bound levels of the lipid peroxidation product, 4-hydroxy-2-nonenal, in brain from persons with mild cognitive impairment." *Neurosci Lett* **397**(3): 170-173.
Calderwood, S. K., S. S. Mambula, P. J. Gray, Jr. and J. R. Theriault (2007). "Extracellular heat shock proteins in cell signaling." *FEBS Lett* **581**(19): 3689-3694.
Ceccariglia, S., A. D'Altocolle, A. Del Fa, F. Pizzolante, E. Caccia, F. Michetti and C. Gangitano (2011). "Cathepsin D plays a crucial role in the trimethyltin-induced hippocampal neurodegeneration process." *Neuroscience* **174**: 160-170.
Chaudhary, P., R. Sharma, A. Sharma, R. Vatsyayan, S. Yadav, S. S. Singhal, N. Rauniyar, L. Prokai, S. Awasthi and Y. C. Awasthi (2010). "Mechanisms of 4-hydroxy-2-nonenal induced pro- and anti-apoptotic signaling." *Biochemistry* **49**(29): 6263-6275.
Chen, X., Q. Tao, H. Yu, L. Zhang and X. Cao (2002). "Tumor cell membrane-bound heat shock protein 70 elicits antitumor immunity." *Immunol Lett* **84**(2): 81-87.
Chi, C., H. Zhu, M. Han, Y. Zhuang, X. Wu and T. Xu (2010). "Disruption of lysosome function promotes tumor growth and metastasis in Drosophila." *J Biol Chem* **285**(28): 21817-21823.
Chong, Z. Z., F. Li and K. Maiese (2005). "Oxidative stress in the brain: novel cellular targets that govern survival during neurodegenerative disease." *Prog Neurobiol* **75**(3): 207-246.
Chtcheglova, L. A. and P. Hinterdorfer (2018). "Simultaneous AFM topography and recognition imaging at the plasma membrane of mammalian cells." *Semin Cell Dev Biol* **73**: 45-56.
Ciocca, D. R. and S. K. Calderwood (2005). "Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications." *Cell Stress Chaperones* **10**(2): 86-103.
Cloutier, P. and B. Coulombe (2013). "Regulation of molecular chaperones through post-translational modifications: decrypting the chaperone code." *Biochim Biophys Acta* **1829**(5): 443-454.
Cordonnier, M., G. Chanteloup, N. Isambert, R. Seigneuric, P. Fumoleau, C. Garrido and J. Gobbo (2017). "Exosomes in cancer theranostic: Diamonds in the rough." *Cell Adh Migr* **11**(2): 151-163.
Crul, T., N. Toth, S. Piotto, P. Literati-Nagy, K. Tory, P. Haldimann, B. Kalmar, L. Greensmith, Z. Torok, G. Balogh, I. Gombos, F. Campana, S. Concilio, F. Gallyas, G. Nagy, Z. Berente, B. Gungor, M. Peter, A. Glatz, A. Hunya, Z. Literati-Nagy, L. Vigh, Jr., F. Hoogstra-Berends, A. Heeres, I. Kuipers, L. Loen, J. P. Seerden, D. Zhang, R. A. Meijering, R. H. Henning, B. J. Brundel, H. H. Kampinga, L. Koranyi, Z. Szilvassy, J. Mandl, B. Sumegi, M. A. Febbraio, I. Horvath, P. L. Hooper and L. Vigh (2013). "Hydroximic acid derivatives: pleiotropic HSP co-inducers restoring homeostasis and robustness." *Curr Pharm Des* **19**(3): 309-346.
Dalleau, S., M. Baradat, F. Gueraud and L. Huc (2013). "Cell death and diseases related to oxidative stress: 4-hydroxynonenal (HNE) in the balance." *Cell Death Differ* **20**(12): 1615-1630.
Daugaard, M., M. Rohde and M. Jaattela (2007). "The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions." *FEBS Lett* **581**(19): 3702-3710.
Doulias, P. T., P. Kotoglou, M. Tenopoulou, D. Keramisanou, T. Tzavaras, U. Brunk, D. Galaris and C. Angelidis (2007). "Involvement of heat shock protein-70 in the mechanism of hydrogen peroxide-induced DNA damage: the role of lysosomes and iron." *Free Radic Biol Med* **42**(4): 567-577.
Dubois, M. F. and O. Bensaude (1993). "MAP kinase activation during heat shock in quiescent and exponentially growing mammalian cells." *FEBS Lett* **324**(2): 191-195.

Ellegaard, A. M., C. Dehlendorff, A. C. Vind, A. Anand, L. Cederkvist, N. H. Petersen, J. Nylandsted, J. Stenvang, A. Mellemgaard, K. Osterlind, S. Friis and M. Jäättelä (2016). "Repurposing Cationic Amphiphilic Antihistamines for Cancer Treatment." *EBioMedicine* **9**: 130-139.
Ellegaard, A. M., L. Groth-Pedersen, V. Oorschot, J. Klumperman, T. Kirkegaard, J. Nylandsted and M. Jäättelä (2013). "Sunitinib and SU11652 Inhibit Acid Sphingomyelinase, Destabilize Lysosomes, and Inhibit Multidrug Resistance." *Mol Cancer Ther* **12**(10): 2018-2030.
Escriba, P. V., X. Busquets, J. Inokuchi, G. Balogh, Z. Torok, I. Horvath, J. L. Harwood and L. Vigh (2015). "Membrane lipid therapy: Modulation of the cell membrane composition and structure as a molecular base for drug discovery and new disease treatment." *Prog Lipid Res* **59**: 38-53.
Eskelinen, E. L., Y. Tanaka and P. Saftig (2003). "At the acidic edge: emerging functions for lysosomal membrane proteins." *Trends Cell Biol* **13**(3): 137-145.
Farkas-Himsley, H., R. Hill, B. Rosen, S. Arab and C. A. Lingwood (1995). "The bacterial colicin active against tumor cells in vitro and in vivo is verotoxin 1." *Proc Natl Acad Sci U S A* **92**(15): 6996-7000.
Feldstein, A. E., N. W. Werneburg, A. Canbay, M. E. Guicciardi, S. F. Bronk, R. Rydzewski, L. J. Burgart and G. J. Gores (2004). "Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway." *Hepatology* **40**(1): 185-194.
Folts, C. J., N. Scott-Hewitt, C. Proschel, M. Mayer-Proschel and M. Noble (2016). "Lysosomal Re-acidification Prevents Lysosphingolipid-Induced Lysosomal Impairment and Cellular Toxicity." *PLoS Biol* **14**(12): e1002583.
Futerman, A. H. and G. van Meer (2004). "The cell biology of lysosomal storage disorders." *Nat Rev Mol Cell Biol* **5**(7): 554-565.
Gabai, V. L., J. A. Yaglom, T. Waldman and M. Y. Sherman (2009). "Heat shock protein Hsp72 controls oncogene-induced senescence pathways in cancer cells." *Mol Cell Biol* **29**(2): 559-569.
Gabai, V. L., J. A. Yaglom, Y. Wang, L. Meng, H. Shao, G. Kim, T. Colvin, J. Gestwicki and M. Y. Sherman (2016). "Anticancer Effects of Targeting Hsp70 in Tumor Stromal Cells." *Cancer Res* **76**(20): 5926-5932.
Gastpar, R., M. Gehrmann, M. A. Bausero, A. Asea, C. Gross, J. A. Schroeder and G. Multhoff (2005). "Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells." *Cancer Res* **65**(12): 5238-5247.
Gehrmann, M., G. Liebisch, G. Schmitz, R. Anderson, C. Steinem, A. De Maio, G. Pockley and G. Multhoff (2008). "Tumor-specific Hsp70 plasma membrane localization is enabled by the glycosphingolipid Gb3." *PLoS One* **3**(4): e1925.
Gehrmann, M., J. Marienhagen, H. Eichholtz-Wirth, E. Fritz, J. Ellwart, M. Jaattela, T. Zilch and G. Multhoff (2005). "Dual function of membrane-bound heat shock protein 70 (Hsp70), Bag-4, and Hsp40: protection against radiation-induced effects and target structure for natural killer cells." *Cell Death Differ* **12**(1): 38-51.
Gehrmann, M., S. Stangl, A. Kirschner, G. A. Foulds, W. Sievert, B. T. Doss, A. Walch, A. G. Pockley and G. Multhoff (2012). "Immunotherapeutic targeting of membrane Hsp70-expressing tumors using recombinant human granzyme B." *PLoS One* **7**(7): e41341.
Gombos, I., T. Crul, S. Piotto, B. Gungor, Z. Torok, G. Balogh, M. Peter, J. P. Slotte, F. Campana, A. M. Pilbat, A. Hunya, N. Toth, Z. Literati-Nagy, L. Vigh, Jr., A. Glatz, M. Brameshuber, G. J. Schutz, A. Hevener, M. A. Febbraio, I. Horvath and L. Vigh (2011). "Membrane-lipid therapy in

operation: the HSP co-inducer BGP-15 activates stress signal transduction pathways by remodeling plasma membrane rafts." *PLoS One* **6**(12): e28818.
Gomez-Sintes, R., M. D. Ledesma and P. Boya (2016). "Lysosomal cell death mechanisms in aging." *Ageing Res Rev* **32**: 150-168.
Gong, J., Y. Zhang, J. Durfee, D. Weng, C. Liu, S. Koido, B. Song, V. Apostolopoulos and S. K. Calderwood (2010). "A heat shock protein 70-based vaccine with enhanced immunogenicity for clinical use." *J Immunol* **184**(1): 488-496.
Gregory, C. D., T. Tursz, C. F. Edwards, C. Tetaud, M. Talbot, B. Caillou, A. B. Rickinson and M. Lipinski (1987). "Identification of a subset of normal B cells with a Burkitt's lymphoma (BL)-like phenotype." *J Immunol* **139**(1): 313-318.
Gross, C., D. Hansch, R. Gastpar and G. Multhoff (2003). "Interaction of heat shock protein 70 peptide with NK cells involves the NK receptor CD94." *Biol Chem* **384**(2): 267-279.
Groth-Pedersen, L. and M. Jäättelä (2013). "Combating apoptosis and multidrug resistant cancers by targeting lysosomes." *Cancer Lett* **332**(2): 265-274.
Groth-Pedersen, L., M. S. Ostenfeld, M. Høyer-Hansen, J. Nylandsted and M. Jäättelä (2007). "Vincristine induces dramatic lysosomal changes and sensitizes cancer cells to lysosome destabilizing siramesine." *Cancer Res* **67**: 2217-2225.
Gungor, B., I. Gombos, T. Crul, F. Ayaydin, L. Szabo, Z. Torok, L. Mates, L. Vigh and I. Horvath (2014). "Rac1 participates in thermally induced alterations of the cytoskeleton, cell morphology and lipid rafts, and regulates the expression of heat shock proteins in B16F10 melanoma cells." *PLoS One* **9**(2): e89136.
Gunther, S., C. Ostheimer, S. Stangl, H. M. Specht, P. Mozes, M. Jesinghaus, D. Vordermark, S. E. Combs, F. Peltz, M. P. Jung and G. Multhoff (2015). "Correlation of Hsp70 Serum Levels with Gross Tumor Volume and Composition of Lymphocyte Subpopulations in Patients with Squamous Cell and Adeno Non-Small Cell Lung Cancer." *Front Immunol* **6**: 556.
Guzhova, I., K. Kislyakova, O. Moskaliova, I. Fridlanskaya, M. Tytell, M. Cheetham and B. Margulis (2001). "In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance." *Brain Res* **914**(1-2): 66-73.
Gyrd-Hansen, M., T. Farkas, N. Fehrenbacher, L. Bastholm, M. Høyer-Hansen, F. Elling, D. Wallach, R. Flavell, G. Kroemer, J. Nylandsted and M. Jäättelä (2006). "Apoptosome-independent activation of lysosomal cell death pathway by caspase-9." *Mol Cell Biol* **26**(21): 7880-7891.
Hageman, J. and H. H. Kampinga (2009). "Computational analysis of the human HSPH/HSPA/DNAJ family and cloning of a human HSPH/HSPA/DNAJ expression library." *Cell Stress Chaperones* **14**(1): 1-21.
Hämälistö, S. and M. Jäättelä (2016). "Lysosomes in cancer-living on the edge (of the cell)." *Curr Opin Cell Biol* **39**: 69-76.
Hantschel, M., K. Pfister, A. Jordan, R. Scholz, R. Andreesen, G. Schmitz, H. Schmetzer, W. Hiddemann and G. Multhoff (2000). "Hsp70 plasma membrane expression on primary tumor biopsy material and bone marrow of leukemic patients." *Cell Stress Chaperones* **5**(5): 438-442.
Hartmann, D., J. Lucks, S. Fuchs, S. Schiffmann, Y. Schreiber, N. Ferreiros, J. Merkens, R. Marschalek, G. Geisslinger and S. Grosch (2012). "Long chain ceramides and very long chain ceramides have opposite effects on human breast and colon cancer cell growth." *Int J Biochem Cell Biol* **44**(4): 620-628.
Heinrich, M., M. Wickel, S. Winoto-Morbach, W. Schneider-Brachert, T. Weber, J. Brunner, P. Saftig, C. Peters, M. Kronke and S. Schutze (2000). "Ceramide as an activator lipid of cathepsin D." *Adv Exp Med Biol* **477**: 305-315.

Hightower, L. E. and P. T. Guidon, Jr. (1989). "Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins." J *Cell Physiol* **138**(2): 257-266.
Hooper, P. L., P. L. Hooper, M. Tytell and L. Vigh (2010). "Xenohormesis: health benefits from an eon of plant stress response evolution." *Cell Stress Chaperones* **15**(6): 761-770.
Horvath, I., A. Glatz, V. Varvasovszki, Z. Torok, T. Pali, G. Balogh, E. Kovacs, L. Nadasdi, S. Benko, F. Joo and L. Vigh (1998). "Membrane physical state controls the signaling mechanism of the heat shock response in Synechocystis PCC 6803: identification of hsp17 as a "fluidity gene"." *Proc Natl Acad Sci U S A* **95**(7): 3513-3518.
Horvath, I., G. Multhoff, A. Sonnleitner and L. Vigh (2008). "Membrane-associated stress proteins: more than simply chaperones." *Biochim Biophys Acta* **1778**(7-8): 1653-1664.
Horvath, I. and L. Vigh (2010). "Cell biology: Stability in times of stress." *Nature* **463**(7280): 436-438.
Hromadnikova, I., S. Li, K. Kotlabova and A. M. Dickinson (2016). "Influence of In Vitro IL-2 or IL-15 Alone or in Combination with Hsp 70 Derived 14-Mer Peptide (TKD) on the Expression of NK Cell Activatory and Inhibitory Receptors on Peripheral Blood T Cells, B Cells and NKT Cells." *PLoS One* **11**(3): e0151535.
Hwang, J. H., J. K. Ryu, Y. B. Yoon, K. H. Lee, Y. S. Park, J. W. Kim, N. Kim, D. H. Lee, J. B. Jeong, J. S. Seo and Y. T. Kim (2005). "Spontaneous activation of pancreas trypsinogen in heat shock protein 70.1 knock-out mice." *Pancreas* **31**(4): 332-336.
Infante, R. E., M. L. Wang, A. Radhakrishnan, H. J. Kwon, M. S. Brown and J. L. Goldstein (2008). "NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid bilayers, a step in cholesterol egress from lysosomes." *Proc Natl Acad Sci U S A* **105**(40): 15287-15292.
Jäättelä, M., D. Wissing, P. A. Bauer and G. C. Li (1992). "Major heat shock protein hsp70 protects tumor cells from tumor necrosis factor cytotoxicity." *EMBO J.* **11**: 3507-3512.
Jäättelä, M., D. Wissing, K. Kokholm, T. Kallunki and M. Egeblad (1998). "Hsp70 exerts its anti-apoptotic function downstream of caspase-3-like proteases." *EMBO J.* **17**: 6124-6134.
Jahchan, N. S., J. T. Dudley, P. K. Mazur, N. Flores, D. Yang, A. Palmerton, A. F. Zmoos, D. Vaka, K. Q. Tran, M. Zhou, K. Krasinska, J. W. Riess, J. W. Neal, P. Khatri, K. S. Park, A. J. Butte and J. Sage (2013). "A drug repositioning approach identifies tricyclic antidepressants as inhibitors of small cell lung cancer and other neuroendocrine tumors." *Cancer Discov* **3**(12): 1364-1377.
Jakobsson, M. E., A. Moen, L. Bousset, W. Egge-Jacobsen, S. Kernstock, R. Melki and P. O. Falnes (2013). "Identification and characterization of a novel human methyltransferase modulating Hsp70 protein function through lysine methylation." *J Biol Chem* **288**(39): 27752-27763.
Jeyakumar, M., R. Thomas, E. Elliot-Smith, D. A. Smith, A. C. van der Spoel, A. D'Azzo, V. H. Perry, T. D. Butters, R. A. Dwek and F. M. Platt (2003). "Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis." *Brain : a journal of neurology* **126**: 974-987.
Johansson, D., A. Johansson, K. Grankvist, U. Andersson, R. Henriksson, P. Bergstrom, T. Brannstrom and P. Behnam-Motlagh (2006). "Verotoxin-1 induction of apoptosis in Gb3-expressing human glioma cell lines." *Cancer Biol Ther* **5**(9): 1211-1217.
Juhasz, K., A. M. Lipp, B. Nimmervoll, A. Sonnleitner, J. Hesse, T. Haselgruebler and Z. Balogi (2013). "The complex function of hsp70 in metastatic cancer." *Cancers (Basel)* **6**(1): 42-66.

Juhasz, K., R. Thuenauer, A. Spachinger, E. Duda, I. Horvath, L. Vigh, A. Sonnleitner and Z. Balogi (2013). "Lysosomal rerouting of Hsp70 trafficking as a potential immune activating tool for targeting melanoma." *Curr Pharm Des* **19**(3): 430-440.
Kågedal, K., M. Zhao, I. Svensson and U. T. Brunk (2001). "Sphingosine-induced apoptosis is dependent on lysosomal proteases." *Biochem J* **359**(Pt 2): 335-343.
Kallunki, T., O. D. Olsen and M. Jäättelä (2013). "Cancer-associated lysosomal changes: friends or foes?" *Oncogene* **32**(16): 1995-2004.
Kasza, A., A. Hunya, Z. Frank, F. Fulop, Z. Torok, G. Balogh, M. Santha, A. Balind, S. Bernath, K. L. Blundell, C. Prodromou, I. Horvath, H. J. Zeiler, P. L. Hooper, L. Vigh and B. Penke (2016). "Dihydropyridine Derivatives Modulate Heat Shock Responses and have a Neuroprotective Effect in a Transgenic Mouse Model of Alzheimer's Disease." *J Alzheimers Dis* **53**(2): 557-571.
Kaur, J., A. Srivastava and R. Ralhan (1998). "Expression of 70-kDa heat shock protein in oral lesions: marker of biological stress or pathogenicity." *Oral Oncol* **34**(6): 496-501.
Keller, J. N., Z. Pang, J. W. Geddes, J. G. Begley, A. Germeyer, G. Waeg and M. P. Mattson (1997). "Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal." *J Neurochem* **69**(1): 273-284.
Kirkegaard, T., J. Gray, D. A. Priestman, K. L. Wallom, J. Atkins, O. D. Olsen, A. Klein, S. Drndarski, N. H. Petersen, L. Ingemann, D. A. Smith, L. Morris, C. Bornaes, S. H. Jorgensen, I. Williams, A. Hinsby, C. Arenz, D. Begley, M. Jaattela and F. M. Platt (2016). "Heat shock protein-based therapy as a potential candidate for treating the sphingolipidoses." *Sci Transl Med* **8**(355): 355ra118.
Kirkegaard, T. and M. Jäättelä (2009). "Lysosomal involvement in cell death and cancer." *Biochim Biophys Acta* **1793**(4): 746-754.
Kirkegaard, T., A. G. Roth, N. H. Petersen, A. K. Mahalka, O. D. Olsen, I. Moilanen, A. Zylicz, J. Knudsen, K. Sandhoff, C. Arenz, P. K. Kinnunen, J. Nylandsted and M. Jaattela (2010). "Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology." *Nature* **463**(7280): 549-553.
Kiselyov, K., G. A. Colletti, A. Terwilliger, K. Ketchum, W. P. Lyons, J. Quinn and S. Muallem (2011). "TRPML: TRansPorters of Metals in Lysosomes essential for cell survival?" *Cell calcium* **50**: 288-294.
Kishimoto, T., R. Ishitsuka and T. Kobayashi (2016). "Detectors for evaluating the cellular landscape of sphingomyelin- and cholesterol-rich membrane domains." *Biochim Biophys Acta* **1861**(8 Pt B): 812-829.
Kollmann, K., M. Damme, S. Markmann, W. Morelle, M. Schweizer, I. Hermans-Borgmeyer, a. K. Röchert, S. Pohl, T. Lübke, J.-C. Michalski, R. Käkelä, S. U. Walkley and T. Braulke (2012). "Lysosomal dysfunction causes neurodegeneration in mucolipidosis II 'knock-in' mice." *Brain : a journal of neurology* **135**: 2661-2675.
Kollmann, K., K. Uusi-Rauva, E. Scifo, J. Tyynelä, A. Jalanko and T. Braulke (2013). "Cell biology and function of neuronal ceroid lipofuscinosis-related proteins." *Biochimica et biophysica acta* **1832**: 1866-1881.
Kolter, T. and K. Sandhoff (2005). "Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids." *Annu Rev Cell Dev Biol* **21**: 81-103.
Kolter, T. and K. Sandhoff (2009). "Lysosomal degradation of membrane lipids." *FEBS Lett.*

Kolzer, M., N. Werth and K. Sandhoff (2004). "Interactions of acid sphingomyelinase and lipid bilayers in the presence of the tricyclic antidepressant desipramine." *FEBS Lett* **559**(1-3): 96-98.
Kopito, R. R. (2000). "Aggresomes, inclusion bodies and protein aggregation." *Trends Cell Biol* **10**(12): 524-530.
Koriyama, Y., K. Sugitani, K. Ogai and S. Kato (2014). "Heat shock protein 70 induction by valproic acid delays photoreceptor cell death by N-methyl-N-nitrosourea in mice." *J Neurochem* **130**(5): 707-719.
Kornhuber, J., P. Tripal, M. Reichel, C. Muhle, C. Rhein, M. Muehlbacher, T. W. Groemer and E. Gulbins (2010). "Functional Inhibitors of Acid Sphingomyelinase (FIASMAs): a novel pharmacological group of drugs with broad clinical applications." *Cell Physiol Biochem* **26**(1): 9-20.
Kurz, T., J. W. Eaton and U. T. Brunk (2010). "Redox activity within the lysosomal compartment: implications for aging and apoptosis." *Antioxidants & redox signaling* **13**: 511-523.
Lamprecht, C., M. Gehrmann, J. Madl, W. Romer, G. Multhoff and A. Ebner (2018). "Molecular AFM imaging of Hsp70-1A association with dipalmitoyl phosphatidylserine reveals membrane blebbing in the presence of cholesterol." *Cell Stress Chaperones*.
Lancaster, G. I. and M. A. Febbraio (2005). "Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins." *J Biol Chem* **280**(24): 23349-23355.
Lazarev, V. F., D. V. Sverchinsky, E. R. Mikhaylova, P. I. Semenyuk, E. Y. Komarova, S. A. Niskanen, A. D. Nikotina, A. V. Burakov, V. G. Kartsev, I. V. Guzhova and B. A. Margulis (2018). "Sensitizing tumor cells to conventional drugs: HSP70 chaperone inhibitors, their selection and application in cancer models." *Cell Death Dis* **9**(2): 41.
Lazaris, A. C., G. E. Theodoropoulos, K. Aroni, A. Saetta and P. S. Davaris (1995). "Immunohistochemical expression of C-myc oncogene, heat shock protein 70 and HLA-DR molecules in malignant cutaneous melanoma." *Virchows Arch* **426**(5): 461-467.
Lee, J. H., M. K. McBrayer, D. M. Wolfe, L. J. Haslett, A. Kumar, Y. Sato, P. P. Lie, P. Mohan, E. E. Coffey, U. Kompella, C. H. Mitchell, E. Lloyd-Evans and R. A. Nixon (2015). "Presenilin 1 Maintains Lysosomal Ca(2+) Homeostasis via TRPML1 by Regulating vATPase-Mediated Lysosome Acidification." *Cell Rep* **12**(9): 1430-1444.
Lee, S., Y. Sato and R. A. Nixon (2011). "Lysosomal proteolysis inhibition selectively disrupts axonal transport of degradative organelles and causes an Alzheimer's-like axonal dystrophy." *J Neurosci* **31**(21): 7817-7830.
Lindberg, A. A., J. E. Brown, N. Stromberg, M. Westling-Ryd, J. E. Schultz and K. A. Karlsson (1987). "Identification of the carbohydrate receptor for Shiga toxin produced by Shigella dysenteriae type 1." *J Biol Chem* **262**(4): 1779-1785.
Lingwood, C. A., H. Law, S. Richardson, M. Petric, J. L. Brunton, S. De Grandis and M. Karmali (1987). "Glycolipid binding of purified and recombinant Escherichia coli produced verotoxin in vitro." *J Biol Chem* **262**(18): 8834-8839.
Linke, T., G. Wilkening, S. Lansmann, H. Moczall, O. Bartelsen, J. Weisgerber and K. Sandhoff (2001). "Stimulation of acid sphingomyelinase activity by lysosomal lipids and sphingolipid activator proteins." *Biol Chem* **382**(2): 283-290.
Linke, T., G. Wilkening, F. Sadeghlar, H. Mozcall, K. Bernardo, E. Schuchman and K. Sandhoff (2001). "Interfacial regulation of acid ceramidase activity. Stimulation of ceramide degradation by lysosomal lipids and sphingolipid activator proteins." *J Biol Chem* **276**(8): 5760-5768.

Lipton, P. (2013). "Lysosomal membrane permeabilization as a key player in brain ischemic cell death: a "lysosomocentric" hypothesis for ischemic brain damage." *Transl Stroke Res* **4**(6): 672-684.
Lloyd-Evans, E. (2016). "Acidic Ca(2+) stores in neurodegeneration." *Messenger (Los Angel)* **5**(1-2): 37-55.
Lloyd-Evans, E. and L. J. Haslett (2016). "The lysosomal storage disease continuum with ageing-related neurodegenerative disease." *Ageing Res Rev* **32**: 104-121.
Lloyd-Evans, E., A. J. Morgan, X. He, D. A. Smith, E. Elliot-Smith, D. J. Sillence, G. C. Churchill, E. H. Schuchman, A. Galione and F. M. Platt (2008). "Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium." *Nat Med* **14**(11): 1247-1255.
Lloyd-Evans, E., H. Waller-Evans, K. Peterneva and F. M. Platt (2010). "Endolysosomal calcium regulation and disease." *Biochem Soc Trans* **38**(6): 1458-1464.
Lopez, V., D. M. Cauvi, N. Arispe and A. De Maio (2016). "Bacterial Hsp70 (DnaK) and mammalian Hsp70 interact differently with lipid membranes." *Cell Stress Chaperones* **21**(4): 609-616.
Lv, L. H., Y. L. Wan, Y. Lin, W. Zhang, M. Yang, G. L. Li, H. M. Lin, C. Z. Shang, Y. J. Chen and J. Min (2012). "Anticancer drugs cause release of exosomes with heat shock proteins from human hepatocellular carcinoma cells that elicit effective natural killer cell antitumor responses in vitro." *J Biol Chem* **287**(19): 15874-15885.
Mahalka, A. K., T. Kirkegaard, L. T. Jukola, M. Jaattela and P. K. Kinnunen (2014). "Human heat shock protein 70 (Hsp70) as a peripheral membrane protein." *Biochim Biophys Acta* **1838**(5): 1344-1361.
Maloney, M. D., B. Binnington-Boyd and C. A. Lingwood (1999). "Globotriaosyl ceramide modulates interferon-alpha-induced growth inhibition and CD19 expression in Burkitt's lymphoma cells." *Glycoconj J* **16**(12): 821-828.
Maloney, M. D. and C. A. Lingwood (1994). "CD19 has a potential CD77 (globotriaosyl ceramide)-binding site with sequence similarity to verotoxin B-subunits: implications of molecular mimicry for B cell adhesion and enterohemorrhagic Escherichia coli pathogenesis." *J Exp Med* **180**(1): 191-201.
Mambula, S. S. and S. K. Calderwood (2006). "Heat shock protein 70 is secreted from tumor cells by a nonclassical pathway involving lysosomal endosomes." *J Immunol* **177**(11): 7849-7857.
Mamelak, D. and C. Lingwood (2001). "The ATPase domain of hsp70 possesses a unique binding specificity for 3'-sulfogalactolipids." *J Biol Chem* **276**(1): 449-456.
Mark, R. J., M. A. Lovell, W. R. Markesbery, K. Uchida and M. P. Mattson (1997). "A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide." *J Neurochem* **68**(1): 255-264.
Matsuo, H., J. Chevallier, N. Mayran, I. Le Blanc, C. Ferguson, J. Faure, N. S. Blanc, S. Matile, J. Dubochet, R. Sadoul, R. G. Parton, F. Vilbois and J. Gruenberg (2004). "Role of LBPA and Alix in multivesicular liposome formation and endosome organization." *Science* **303**(5657): 531-534.
Mattson, M. P. (2009). "Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders." *Exp Gerontol* **44**(10): 625-633.
Mena, S., M. L. Rodriguez, X. Ponsoda, J. M. Estrela, M. Jäättelä and A. L. Ortega (2012). "Pterostilbene-induced tumor cytotoxicity: a lysosomal membrane permeabilization-dependent mechanism." *PLoS One* **7**(9): e44524.

Metkar, S. S., B. Wang, E. Catalan, G. Anderluh, R. J. Gilbert, J. Pardo and C. J. Froelich (2011). "Perforin rapidly induces plasma membrane phospholipid flip-flop." *PLoS One* **6**(9): e24286.
Micsenyi, M. C., J. Sikora, G. Stephney, K. Dobrenis and S. U. Walkley (2013). "Lysosomal membrane permeability stimulates protein aggregate formation in neurons of a lysosomal disease." *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**: 10815-10827.
Morgner, N., C. Schmidt, V. Beilsten-Edmands, I. O. Ebong, N. A. Patel, E. M. Clerico, E. Kirschke, S. Daturpalli, S. E. Jackson, D. Agard and C. V. Robinson (2015). "Hsp70 forms antiparallel dimers stabilized by post-translational modifications to position clients for transfer to Hsp90." *Cell Rep* **11**(5): 759-769.
Morimoto, R. I. (1993). "Cells in stress: transcriptional activation of heat shock genes." *Science* **259**(5100): 1409-1410.
Morozova, K., C. C. Clement, S. Kaushik, B. Stiller, E. Arias, A. Ahmad, J. N. Rauch, V. Chatterjee, C. Melis, B. Scharf, J. E. Gestwicki, A. M. Cuervo, E. R. Zuiderweg and L. Santambrogio (2016). "Structural and Biological Interaction of hsc-70 Protein with Phosphatidylserine in Endosomal Microautophagy." *J Biol Chem* **291**(35): 18096-18106.
Mu, T.-w., D. S. T. Ong, Y.-j. Wang, W. E. Balch, J. R. Yates, L. Segatori and J. W. Kelly (2008). "Chemical and biological approaches synergize to ameliorate protein-folding diseases." Cell **134**: 769-781.
Multhoff, G., C. Botzler and R. Issels (1998). "The role of heat shock proteins in the stimulation of an immune response." *Biol Chem* **379**(3): 295-300.
Multhoff, G., C. Botzler, L. Jennen, J. Schmidt, J. Ellwart and R. Issels (1997). "Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells." *J Immunol* **158**(9): 4341-4350.
Multhoff, G., C. Botzler, M. Wiesnet, E. Muller, T. Meier, W. Wilmanns and R. D. Issels (1995). "A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor cells, but not on normal cells." *Int J Cancer* **61**(2): 272-279.
Multhoff, G., L. Mizzen, C. C. Winchester, C. M. Milner, S. Wenk, G. Eissner, H. H. Kampinga, B. Laumbacher and J. Johnson (1999). "Heat shock protein 70 (Hsp70) stimulates proliferation and cytolytic activity of natural killer cells." *Exp Hematol* **27**(11): 1627-1636.
Multhoff, G., K. Pfister, C. Botzler, A. Jordan, R. Scholz, H. Schmetzer, R. Burgstahler and W. Hiddemann (2000). "Adoptive transfer of human natural killer cells in mice with severe combined immunodeficiency inhibits growth of Hsp70-expressing tumors." *Int J Cancer* **88**(5): 791-797.
Multhoff, G., K. Pfister, M. Gehrmann, M. Hantschel, C. Gross, M. Hafner and W. Hiddemann (2001). "A 14-mer Hsp70 peptide stimulates natural killer (NK) cell activity." *Cell Stress Chaperones* **6**(4): 337-344.
Murakami, N., A. Kuhnel, T. E. Schmid, K. Ilicic, S. Stangl, I. S. Braun, M. Gehrmann, M. Molls, J. Itami and G. Multhoff (2015). "Role of membrane Hsp70 in radiation sensitivity of tumor cells." *Radiat Oncol* **10**: 149.
Nagy, E., Z. Balogi, I. Gombos, M. Akerfelt, A. Bjorkbom, G. Balogh, Z. Torok, A. Maslyanko, A. Fiszer-Kierzkowska, K. Lisowska, P. J. Slotte, L. Sistonen, I. Horvath and L. Vigh (2007). "Hyperfluidization-coupled membrane microdomain reorganization is linked to activation of the heat shock response in a murine melanoma cell line." *Proc Natl Acad Sci U S A* **104**(19): 7945-7950.
Nakasone, N., Y. S. Nakamura, K. Higaki, N. Oumi, K. Ohno and H. Ninomiya (2014). "Endoplasmic Reticulum-associated Degradation of Niemann-Pick C1: EVIDENCE FOR THE

ROLE OF HEAT SHOCK PROTEINS AND IDENTIFICATION OF LYSINE RESIDUES THAT ACCEPT UBIQUITIN." *The Journal of biological chemistry* **289**: 19714-19725.
Nanbu, K., I. Konishi, M. Mandai, H. Kuroda, A. A. Hamid, T. Komatsu and T. Mori (1998). "Prognostic significance of heat shock proteins HSP70 and HSP90 in endometrial carcinomas." *Cancer Detect Prev* **22**(6): 549-555.
Nimmervoll, B., L. A. Chtcheglova, K. Juhasz, N. Cremades, F. A. Aprile, A. Sonnleitner, P. Hinterdorfer, L. Vigh, J. Preiner and Z. Balogi (2015). "Cell surface localised Hsp70 is a cancer specific regulator of clathrin-independent endocytosis." *FEBS Lett* **589**(19 Pt B): 2747-2753.
Nixon, R. A., J. Wegiel, A. Kumar, W. H. Yu, C. Peterhoff, A. Cataldo and A. M. Cuervo (2005). "Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study." *J Neuropathol Exp Neurol* **64**(2): 113-122.
Nollen, E. A., F. A. Salomons, J. F. Brunsting, J. J. van der Want, O. C. Sibon and H. H. Kampinga (2001). "Dynamic changes in the localization of thermally unfolded nuclear proteins associated with chaperone-dependent protection." *Proc Natl Acad Sci U S A* **98**(21): 12038-12043.
Novak, A., B. Binnington, B. Ngan, K. Chadwick, N. Fleshner and C. A. Lingwood (2013). "Cholesterol masks membrane glycosphingolipid tumor-associated antigens to reduce their immunodetection in human cancer biopsies." *Glycobiology* **23**(11): 1230-1239.
Nudelman, I. L., A. A. Deutsch and R. Reiss (1987). "Primary hyperparathyroidism due to mediastinal parathyroid adenoma." *Int Surg* **72**(2): 104-108.
Nutikka, A. and C. Lingwood (2004). "Generation of receptor-active, globotriaosyl ceramide/cholesterol lipid 'rafts' in vitro : A new assay to define factors affecting glycosphingolipid receptor activity." *Glycoconj J* **20**(1): 33-38.
Nylandsted, J., M. Gyrd-Hansen, A. Danielewicz, N. Fehrenbacher, U. Lademann, M. Hoyer-Hansen, E. Weber, G. Multhoff, M. Rohde and M. Jaattela (2004). "Heat shock protein 70 promotes cell survival by inhibiting lysosomal membrane permeabilization." J *Exp Med* **200**(4): 425-435.
Nylandsted, J., M. Jäättelä, E. K. Hoffmann and S. F. Pedersen (2004). "Heat shock protein 70 inhibits shrinkage-induced programmed cell death via mechanisms independent of effects on cell volume-regulatory membrane transport proteins." *Pflugers Arch* **449**(2): 175-185.
O'Leary, E. M. and S. a. Igdoura (2012). "The therapeutic potential of pharmacological chaperones and proteosomal inhibitors, Celastrol and MG132 in the treatment of sialidosis." *Molecular genetics and metabolism* **107**: 173-185.
Oberoi, P., R. A. Jabulowsky, H. Bahr-Mahmud and W. S. Wels (2013). "EGFR-targeted granzyme B expressed in NK cells enhances natural cytotoxicity and mediates specific killing of tumor cells." *PLoS One* **8**(4): e61267.
Oikawa, S., T. Yamada, T. Minohata, H. Kobayashi, A. Furukawa, S. Tada-Oikawa, Y. Hiraku, M. Murata, M. Kikuchi and T. Yamashima (2009). "Proteomic identification of carbonylated proteins in the monkey hippocampus after ischemia-reperfusion." *Free Radic Biol Med* **46**(11): 1472-1477.
Olson, O. C. and J. A. Joyce (2015). "Cysteine cathepsin proteases: regulators of cancer progression and therapeutic response." *Nat Rev Cancer* **15**(12): 712-729.
Oninla, V. O., B. Breiden, J. O. Babalola and K. Sandhoff (2014). "Acid sphingomyelinase activity is regulated by membrane lipids and facilitates cholesterol transfer by NPC2." *J Lipid Res* **55**(12): 2606-2619.
Ostenfeld, M. S., M. Høyer-Hansen, L. Bastholm, N. Fehrenbacher, O. D. Olsen, L. Groth-Pedersen, P. Puustinen, T. Kirkegaard-Sorensen, J. Nylandsted, T. Farkas and M. Jäättelä (2008).

"Anti-cancer agent siramesine is a lysosomotropic detergent that induces cytoprotective autophagosome accumulation." *Autophagy* **4**(4): 487-499.
Peinado, H., S. Lavotshkin and D. Lyden (2011). "The secreted factors responsible for pre-metastatic niche formation: old sayings and new thoughts." *Semin Cancer Biol* **21**(2): 139-146.
Penke, B., F. Bogar, T. Crul, M. Santha, M. E. Toth and L. Vigh (2018). "Heat Shock Proteins and Autophagy Pathways in Neuroprotection: from Molecular Bases to Pharmacological Interventions." *Int J Mol Sci* **19**(1).
Penke, B., G. Paragi, J. Gera, R. Berkecz, Z. Kovacs, T. Crul and L. Vigh (2018). "The Role of Lipids and Membranes in the Pathogenesis of Alzheimer's Disease: A Comprehensive View." *Curr Alzheimer Res.*
Perluigi, M., R. Coccia and D. A. Butterfield (2012). "4-Hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neurodegenerative diseases: a toxic combination illuminated by redox proteomics studies." *Antioxid Redox Signal* **17**(11): 1590-1609.
Petersen, N. H. and T. Kirkegaard (2010). "HSP70 and lysosomal storage disorders: novel therapeutic opportunities." *Biochem Soc Trans* **38**(6): 1479-1483.
Petersen, N. H., T. Kirkegaard, O. D. Olsen and M. Jaattela (2010). "Connecting Hsp70, sphingolipid metabolism and lysosomal stability." *Cell Cycle* **9**(12): 2305-2309.
Petersen, N. H., O. D. Olsen, L. Groth-Pedersen, A. M. Ellegaard, M. Bilgin, S. Redmer, M. S. Ostenfeld, D. Ulanet, T. H. Dovmark, A. Lonborg, S. D. Vindelov, D. Hanahan, C. Arenz, C. S. Ejsing, T. Kirkegaard, M. Rohde, J. Nylandsted and M. Jäättelä (2013). "Transformation-associated changes in sphingolipid metabolism sensitize cells to lysosomal cell death induced by inhibitors of acid sphingomyelinase." *Cancer Cell* **24**(3): 379-393.
Pockley, A. G. (2003). "Heat shock proteins as regulators of the immune response." Lancet **362**(9382): 469-476.
Pockley, A. G., J. Shepherd and J. M. Corton (1998). "Detection of heat shock protein 70 (Hsp70) and anti-Hsp70 antibodies in the serum of normal individuals." *Immunol Invest* **27**(6): 367-377.
Rammer, P., L. Groth-Pedersen, T. Kirkegaard, M. Daugaard, A. Rytter, P. Szyniarowski, M. Høyer-Hansen, L. K. Povlsen, J. Nylandsted, J. E. Larsen and M. Jäättelä (2010). "BAMLET activates a lysosomal cell death program in cancer cells." *Mol Cancer Ther* **9**(1): 24-32.
Rankin, E. B. and A. J. Giaccia (2016). "Hypoxic control of metastasis." Science **352**(6282): 175-180.
Roe, M. S., B. Wahab, Z. Torok, I. Horvath, L. Vigh and C. Prodromou (2018). "Dihydropyridines Allosterically Modulate Hsp90 Providing a Novel Mechanism for Heat Shock Protein Co-induction and Neuroprotection." *Front Mol Biosci* **5**: 51.
Rudd, A. K. and N. K. Devaraj (2018). "Traceless synthesis of ceramides in living cells reveals saturation-dependent apoptotic effects." *Proc Natl Acad Sci U S A* **115**(29): 7485-7490.
Saftig, P. and J. Klumperman (2009). "Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function." *Nat Rev Mol Cell Biol* **10**(9): 623-635.
Sahara, S. and T. Yamashima (2010). "Calpain-mediated Hsp70.1 cleavage in hippocampal CA1 neuronal death." *Biochem Biophys Res Commun* **393**(4): 806-811.
Santarosa, M., D. Favaro, M. Quaia and E. Galligioni (1997). "Expression of heat shock protein 72 in renal cell carcinoma: possible role and prognostic implications in cancer patients." *Eur J Cancer* **33**(6): 873-877.
Schaur, R. J., W. Siems, N. Bresgen and P. M. Eckl (2015). "4-Hydroxy-nonenal-A Bioactive Lipid Peroxidation Product." *Biomolecules* **5**(4): 2247-2337.

Schilling, D., M. Gehrmann, C. Steinem, A. De Maio, A. G. Pockley, M. Abend, M. Molls and G. Multhoff (2009). "Binding of heat shock protein 70 to extracellular phosphatidylserine promotes killing of normoxic and hypoxic tumor cells." *FASEB J* **23**(8): 2467-2477.
Schlecht, R., S. R. Scholz, H. Dahmen, A. Wegener, C. Sirrenberg, D. Musil, J. Bomke, H. M. Eggenweiler, M. P. Mayer and B. Bukau (2013). "Functional analysis of Hsp70 inhibitors." *PLoS One* **8**(11): e78443.
Schulze, H., T. Kolter and K. Sandhoff (2009). "Principles of lysosomal membrane degradation: Cellular topology and biochemistry of lysosomal lipid degradation." *Biochim Biophys Acta* **1793**(4): 674-683.
Settembre, C., A. Fraldi, D. L. Medina and A. Ballabio (2013). "Signals from the lysosome: a control centre for cellular clearance and energy metabolism." *Nat Rev Mol Cell Biol* **14**(5): 283-296.
Sezgin, E., I. Levental, S. Mayor and C. Eggeling (2017). "The mystery of membrane organization: composition, regulation and roles of lipid rafts." *Nat Rev Mol Cell Biol* **18**(6): 361-374.
Shchors, K., A. Massaras and D. Hanahan (2015). "Dual Targeting of the Autophagic Regulatory Circuitry in Gliomas with Repurposed Drugs Elicits Cell-Lethal Autophagy and Therapeutic Benefit." *Cancer Cell* **28**(4): 456-471.
Shen, J.-S. (2008). "Globotriaosylceramide induces oxidative stress and up-regulates cell adhesion molecule expression in Fabry disease endothelial cells." *Mol.Genet.Metab* **95**: 163-168.
Sherman, M. Y. and V. L. Gabai (2015). "Hsp70 in cancer: back to the future." *Oncogene* **34**(32): 4153-4161.
Shevtsov, M. A., E. Y. Komarova, D. A. Meshalkina, N. V. Bychkova, N. D. Aksenov, S. V. Abkin, B. A. Margulis and I. V. Guzhova (2014). "Exogenously delivered heat shock protein 70 displaces its endogenous analogue and sensitizes cancer cells to lymphocytes-mediated cytotoxicity." *Oncotarget* **5**(10): 3101-3114.
Shevtsov, M. A., A. V. Pozdnyakov, A. L. Mikhrina, L. Y. Yakovleva, B. P. Nikolaev, A. V. Dobrodumov, E. Y. Komarova, D. A. Meshalkina, A. M. Ischenko, E. Pitkin, I. V. Guzhova and B. A. Margulis (2014). "Effective immunotherapy of rat glioblastoma with prolonged intratumoral delivery of exogenous heat shock protein Hsp70." *Int J Cancer* **135**(9): 2118-2128.
Shin, B. K., H. Wang, A. M. Yim, F. Le Naour, F. Brichory, J. H. Jang, R. Zhao, E. Puravs, J. Tra, C. W. Michael, D. E. Misek and S. M. Hanash (2003). "Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function." *J Biol Chem* **278**(9): 7607-7616.
Silveira, C. P., A. C. Piffer, L. Kmetzsch, F. L. Fonseca, D. A. Soares, C. C. Staats, M. L. Rodrigues, A. Schrank and M. H. Vainstein (2013). "The heat shock protein (Hsp) 70 of Cryptococcus neoformans is associated with the fungal cell surface and influences the interaction between yeast and host cells." *Fungal Genet Biol* **60**: 53-63.
Sonnino, S. and A. Prinetti (2013). "Membrane domains and the "lipid raft" concept." *Curr Med Chem* **20**(1): 4-21.
Soss, S. E., K. L. Rose, S. Hill, S. Jouan and W. J. Chazin (2015). "Biochemical and Proteomic Analysis of Ubiquitination of Hsc70 and Hsp70 by the E3 Ligase CHIP." *PLoS One* **10**(5): e0128240.
Specht, H. M., N. Ahrens, C. Blankenstein, T. Duell, R. Fietkau, U. S. Gaipl, C. Gunther, S. Gunther, G. Habl, H. Hautmann, M. Hautmann, R. M. Huber, M. Molls, R. Offner, C. Rodel, F. Rodel, M. Schutz, S. E. Combs and G. Multhoff (2015). "Heat Shock Protein 70 (Hsp70) Peptide Activated Natural Killer (NK) Cells for the Treatment of Patients with Non-Small Cell Lung

Cancer (NSCLC) after Radiochemotherapy (RCTx) - From Preclinical Studies to a Clinical Phase II Trial." *Front Immunol* **6**: 162.
Srivastava, P. (2002). "Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses." *Annu Rev Immunol* **20**: 395-425.
Stangl, S., M. Gehrmann, J. Riegger, K. Kuhs, I. Riederer, W. Sievert, K. Hube, R. Mocikat, R. Dressel, E. Kremmer, A. G. Pockley, L. Friedrich, L. Vigh, A. Skerra and G. Multhoff (2011). "Targeting membrane heat-shock protein 70 (Hsp70) on tumors by cmHsp70.1 antibody." Proc *Natl Acad Sci U S A* **108**(2): 733-738.
Stiban, J. and M. Perera (2015). "Very long chain ceramides interfere with C16-ceramide-induced channel formation: A plausible mechanism for regulating the initiation of intrinsic apoptosis." *Biochim Biophys Acta* **1848**(2): 561-567.
Stoka, V., V. Turk and B. Turk (2016). "Lysosomal cathepsins and their regulation in aging and neurodegeneration." *Ageing Res Rev* **32**: 22-37.
Subrizi, A., E. Toropainen, E. Ramsay, A. J. Airaksinen, K. Kaarniranta and A. Urtti (2015). "Oxidative stress protection by exogenous delivery of rhHsp70 chaperone to the retinal pigment epithelium (RPE), a possible therapeutic strategy against RPE degeneration." *Pharmaceutical research* **32**: 211-221.
Sukhai, M. A., S. Prabha, R. Hurren, A. C. Rutledge, A. Y. Lee, S. Sriskanthadevan, H. Sun, X. Wang, M. Skrtic, A. Seneviratne, M. Cusimano, B. Jhas, M. Gronda, N. Maclean, E. E. Cho, P. A. Spagnuolo, S. Sharmeen, M. Gebbia, M. Urbanus, K. Eppert, D. Dissanayake, A. Jonet, A. Dassonville-Klimpt, X. Li, A. Datti, P. S. Ohashi, J. Wrana, I. Rogers, P. Sonnet, W. Y. Ellis, S. J. Corey, C. Eaves, M. D. Minden, J. C. Wang, J. E. Dick, C. Nislow, G. Giaever and A. D. Schimmer (2013). "Lysosomal disruption preferentially targets acute myeloid leukemia cells and progenitors." *J Clin Invest* **123**(1): 315-328.
Sullivan, L. C., C. S. Clements, T. Beddoe, D. Johnson, H. L. Hoare, J. Lin, T. Huyton, E. J. Hopkins, H. H. Reid, M. C. Wilce, J. Kabat, F. Borrego, J. E. Coligan, J. Rossjohn and A. G. Brooks (2007). "The heterodimeric assembly of the CD94-NKG2 receptor family and implications for human leukocyte antigen-E recognition." *Immunity* **27**(6): 900-911.
Sultana, R., M. Perluigi, S. F. Newman, W. M. Pierce, C. Cini, R. Coccia and D. A. Butterfield (2010). "Redox proteomic analysis of carbonylated brain proteins in mild cognitive impairment and early Alzheimer's disease." *Antioxid Redox Signal* **12**(3): 327-336.
Syntichaki, P., K. Xu, M. Driscoll and N. Tavernarakis (2002). "Specific aspartyl and calpain proteases are required for neurodegeneration in C. elegans." *Nature* **419**(6910): 939-944.
Syrigos, K. N., K. J. Harrington, A. J. Karayiannakis, E. Sekara, E. Chatziyianni, E. I. Syrigou and J. Waxman (2003). "Clinical significance of heat shock protein-70 expression in bladder cancer." *Urology* **61**(3): 677-680.
Taylor, I. R., B. M. Dunyak, T. Komiyama, H. Shao, X. Ran, V. A. Assimon, C. Kalyanaraman, J. N. Rauch, M. P. Jacobson, E. R. P. Zuiderweg and J. E. Gestwicki (2018). "High-throughput screen for inhibitors of protein-protein interactions in a reconstituted heat shock protein 70 (Hsp70) complex." *J Biol Chem* **293**(11): 4014-4025.
Te Vruchte, D., A. O. Speak, K. L. Wallom, N. Al Eisa, D. A. Smith, C. J. Hendriksz, L. Simmons, R. H. Lachmann, A. Cousins, R. Hartung, E. Mengel, H. Runz, M. Beck, Y. Amraoui, J. Imrie, E. Jacklin, K. Riddick, N. M. Yanjanin, C. A. Wassif, A. Rolfs, F. Rimmele, N. Wright, C. Taylor, U. Ramaswami, T. M. Cox, C. Hastings, X. Jiang, R. Sidhu, D. S. Ory, B. Arias, M. Jeyakumar, D. J. Sillence, J. E. Wraith, F. D. Porter, M. Cortina-Borja and F. M. Platt (2014). "Relative acidic

compartment volume as a lysosomal storage disorder-associated biomarker." *The Journal of clinical investigation*: 1-9.
Teres, S., V. Llado, M. Higuera, G. Barcelo-Coblijn, M. L. Martin, M. A. Noguera-Salva, A. Marcilla-Etxenike, J. M. Garcia-Verdugo, M. Soriano-Navarro, C. Saus, U. Gomez-Pinedo, X. Busquets and P. V. Escriba (2012). "2-Hydroxyoleate, a nontoxic membrane binding anticancer drug, induces glioma cell differentiation and autophagy." *Proc Natl Acad Sci U S A* **109**(22): 8489-8494.
Theriault, J. R., S. S. Mambula, T. Sawamura, M. A. Stevenson and S. K. Calderwood (2005). "Extracellular HSP70 binding to surface receptors present on antigen presenting cells and endothelial/epithelial cells." *FEBS Lett* **579**(9): 1951-1960.
Thuringer, D., K. Berthenet, L. Cronier, G. Jego, E. Solary and C. Garrido (2015). "Oncogenic extracellular HSP70 disrupts the gap-junctional coupling between capillary cells." *Oncotarget* **6**(12): 10267-10283.
Torok, Z., T. Crul, B. Maresca, G. J. Schutz, F. Viana, L. Dindia, S. Piotto, M. Brameshuber, G. Balogh, M. Peter, A. Porta, A. Trapani, I. Gombos, A. Glatz, B. Gungor, B. Peksel, L. Vigh, Jr., B. Csoboz, I. Horvath, M. M. Vijayan, P. L. Hooper, J. L. Harwood and L. Vigh (2014). "Plasma membranes as heat stress sensors: from lipid-controlled molecular switches to therapeutic applications." *Biochim Biophys Acta* **1838**(6): 1594-1618.
Torok, Z., P. Goloubinoff, I. Horvath, N. M. Tsvetkova, A. Glatz, G. Balogh, V. Varvasovszki, D. A. Los, E. Vierling, J. H. Crowe and L. Vigh (2001). "Synechocystis HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding." *Proc Natl Acad Sci U S A* **98**(6): 3098-3103.
Torok, Z., I. Horvath, P. Goloubinoff, E. Kovacs, A. Glatz, G. Balogh and L. Vigh (1997). "Evidence for a lipochaperonin: association of active protein-folding GroESL oligomers with lipids can stabilize membranes under heat shock conditions." *Proc Natl Acad Sci U S A* **94**(6): 2192-2197.
Torok, Z., N. M. Tsvetkova, G. Balogh, I. Horvath, E. Nagy, Z. Penzes, J. Hargitai, O. Bensaude, P. Csermely, J. H. Crowe, B. Maresca and L. Vigh (2003). "Heat shock protein coinducers with no effect on protein denaturation specifically modulate the membrane lipid phase." *Proc Natl Acad Sci U S A* **100**(6): 3131-3136.
Trapp, S., G. R. Rosania, R. W. Horobin and J. Kornhuber (2008). "Quantitative modeling of selective lysosomal targeting for drug design." *Eur Biophys J* **37**(8): 1317-1328.
Triantafilou, M., K. Miyake, D. T. Golenbock and K. Triantafilou (2002). "Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation." *J Cell Sci* **115**(Pt 12): 2603-2611.
Tsuneki, M., S. Maruyama, M. Yamazaki, B. Xu, A. Essa, T. Abe, H. Babkair, J. Cheng, T. Yamamoto and T. Saku (2013). "Extracellular heat shock protein A9 is a novel interaction partner of podoplanin in oral squamous cell carcinoma cells." *Biochem Biophys Res Commun* **434**(1): 124-130.
Tsvetkova, N. M., I. Horvath, Z. Torok, W. F. Wolkers, Z. Balogi, N. Shigapova, L. M. Crowe, F. Tablin, E. Vierling, J. H. Crowe and L. Vigh (2002). "Small heat-shock proteins regulate membrane lipid polymorphism." *Proc Natl Acad Sci U S A* **99**(21): 13504-13509.
Udono, H. and P. K. Srivastava (1993). "Heat shock protein 70-associated peptides elicit specific cancer immunity." *J Exp Med* **178**(4): 1391-1396.

Uittenbogaard, A., Y. Ying and E. J. Smart (1998). "Characterization of a cytosolic heat-shock protein-caveolin chaperone complex. Involvement in cholesterol trafficking." *J Biol Chem* **273**(11): 6525-6532.
van Blitterswijk, W. J., A. H. van der Luit, R. J. Veldman, M. Verheij and J. Borst (2003). "Ceramide: second messenger or modulator of membrane structure and dynamics?" *Biochem J* **369**(Pt 2): 199-211.
van den Eijnde, S. M., L. Boshart, E. H. Baehrecke, C. I. De Zeeuw, C. P. Reutelingsperger and C. Vermeij-Keers (1998). "Cell surface exposure of phosphatidylserine during apoptosis is phylogenetically conserved." *Apoptosis* **3**(1): 9-16.
van Engeland, M., L. J. Nieland, F. C. Ramaekers, B. Schutte and C. P. Reutelingsperger (1998). "Annexin V-affinity assay: a review on an apoptosis detection system based on phosphatidylserine exposure." *Cytometry* **31**(1): 1-9.
VanWinkle, W. B., M. Snuggs, J. C. Miller and L. M. Buja (1994). "Cytoskeletal alterations in cultured cardiomyocytes following exposure to the lipid peroxidation product, 4-hydroxynonenal." *Cell Motil Cytoskeleton* **28**(2): 119-134.
Vicencio, J. M., D. M. Yellon, V. Sivaraman, D. Das, C. Boi-Doku, S. Arjun, Y. Zheng, J. A. Riquelme, J. Kearney, V. Sharma, G. Multhoff, A. R. Hall and S. M. Davidson (2015). "Plasma exosomes protect the myocardium from ischemia-reperfusion injury." *J Am Coll Cardiol* **65**(15): 1525-1536.
Vigh, L., I. Horvath, B. Maresca and J. L. Harwood (2007). "Can the stress protein response be controlled by 'membrane-lipid therapy'?" *Trends Biochem Sci* **32**(8): 357-363.
Vigh, L., B. Maresca and J. L. Harwood (1998). "Does the membrane's physical state control the expression of heat shock and other genes?" *Trends Biochem Sci* **23**(10): 369-374.
Villalpando Rodriguez, G. E. and A. Torriglia (2013). "Calpain 1 induce lysosomal permeabilization by cleavage of lysosomal associated membrane protein 2." *Biochim Biophys Acta* **1833**(10): 2244-2253.
Vitner, E. B., T. Farfel-Becker, R. Eilam, I. Biton and A. H. Futerman (2012). "Contribution of brain inflammation to neuronal cell death in neuronopathic forms of Gaucher's disease." *Brain : a journal of neurology* **135**: 1724-1735.
Wang, J. Q., J. Kon, C. Mogi, M. Tobo, A. Damirin, K. Sato, M. Komachi, E. Malchinkhuu, N. Murata, T. Kimura, A. Kuwabara, K. Wakamatsu, H. Koizumi, T. Uede, G. Tsujimoto, H. Kurose, T. Sato, A. Harada, N. Misawa, H. Tomura and F. Okajima (2004). "TDAG8 is a proton-sensing and psychosine-sensitive G-protein-coupled receptor." *J Biol Chem* **279**(44): 45626-45633.
Xie, K. and S. Huang (2003). "Regulation of cancer metastasis by stress pathways." *Clin Exp Metastasis* **20**(1): 31-43.
Yaglom, J. A., Y. Wang, A. Li, Z. Li, S. Monti, I. Alexandrov, X. Lu and M. Y. Sherman (2018). "Cancer cell responses to Hsp70 inhibitor JG-98: Comparison with Hsp90 inhibitors and finding synergistic drug combinations." *Sci Rep* **8**(1): 3010.
Yamashima, T. (2000). "Implication of cysteine proteases calpain, cathepsin and caspase in ischemic neuronal death of primates." *Prog Neurobiol* **62**(3): 273-295.
Yamashima, T. (2012). "Hsp70.1 and related lysosomal factors for necrotic neuronal death." *J Neurochem* **120**(4): 477-494.
Yamashima, T. (2013). "Reconsider Alzheimer's disease by the 'calpain-cathepsin hypothesis'--a perspective review." *Prog Neurobiol* **105**: 1-23.
Yamashima, T. (2016). "Can 'calpain-cathepsin hypothesis' explain Alzheimer neuronal death?" *Ageing Res Rev* **32**: 169-179.

Yamashima, T., Y. Kohda, K. Tsuchiya, T. Ueno, J. Yamashita, T. Yoshioka and E. Kominami (1998). "Inhibition of ischaemic hippocampal neuronal death in primates with cathepsin B inhibitor CA-074: a novel strategy for neuroprotection based on 'calpain-cathepsin hypothesis'." *Eur J Neurosci* **10**(5): 1723-1733.
Yamashima, T. and S. Oikawa (2009). "The role of lysosomal rupture in neuronal death." *Prog Neurobiol* **89**(4): 343-358.
Yamashima, T., T. C. Saido, M. Takita, A. Miyazawa, J. Yamano, A. Miyakawa, H. Nishijyo, J. Yamashita, S. Kawashima, T. Ono and T. Yoshioka (1996). "Transient brain ischaemia provokes Ca2+, PIP2 and calpain responses prior to delayed neuronal death in monkeys." *Eur J Neurosci* **8**(9): 1932-1944.
Yang, C., C. L. Swallows, C. Zhang, J. Lu, H. Xiao, R. O. Brady and Z. Zhuang (2014). "Celastrol increases glucocerebrosidase activity in Gaucher disease by modulating molecular chaperones." *Proceedings of the National Academy of Sciences of the United States of America* **111**: 249-254.
Yang, M., Z. Xu, Q. Wang, A. Q. Zhang and J. Min (2015). "A hyposensitive anticancer drug induces higher surface expression and release of heat shock proteins in a human hepatocellular carcinoma cell line." *Mol Med Rep* **12**(2): 2879-2885.
Yashin, D. V., E. A. Romanova, O. K. Ivanova and L. P. Sashchenko (2016). "The Tag7-Hsp70 cytotoxic complex induces tumor cell necroptosis via permeabilisation of lysosomes and mitochondria." *Biochimie* **123**: 32-36.
Yeung, T., G. E. Gilbert, J. Shi, J. Silvius, A. Kapus and S. Grinstein (2008). "Membrane phosphatidylserine regulates surface charge and protein localization." *Science* **319**(5860): 210-213.
Zampieri, S., S. H. Mellon, T. D. Butters, M. Nevyjel, F. Douglas, M. Metaboliche and I. B. Garofolo (2009). "Oxidative stress in NPC1 deficient cells: Protective effect of allopregnanolone." *J.Cell Mol.Med.* **13**: 3786-3796.
Zech, T., C. S. Ejsing, K. Gaus, B. de Wet, A. Shevchenko, K. Simons and T. Harder (2009). "Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signalling." *EMBO J* **28**(5): 466-476.
Zhang, G., Y.-P. Yi and G.-J. Zhang (2006). "Effects of arachidonic acid on the lysosomal ion permeability and osmotic stability." *Journal of bioenergetics and biomembranes* **38**: 75-82.
Zhang, H., J. Amick, R. Chakravarti, S. Santarriaga, S. Schlanger, C. McGlone, M. Dare, J. C. Nix, K. M. Scaglione, D. J. Stuehr, S. Misra and R. C. Page (2015). "A bipartite interaction between Hsp70 and CHIP regulates ubiquitination of chaperoned client proteins." *Structure* **23**(3): 472-482.
Zhang, M., D. Wang, P. Li, C. Sun, R. Xu, Z. Geng, W. Xu and Z. Dai (2018). "Interaction of Hsp90 with phospholipid model membranes." *Biochim Biophys Acta* **1860**(2): 611-616.
Zhu, H., T. Yoshimoto and T. Yamashima (2014). "Heat shock protein 70.1 (Hsp70.1) affects neuronal cell fate by regulating lysosomal acid sphingomyelinase." *J Biol Chem* **289**(40): 27432-27443.
Zunino, B. and J. E. Ricci (2016). "Hyperthermic intra-peritoneal chemotherapy and anticancer immune response." *Oncoimmunology* **5**(1): e1060392.